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NEURAL CIRCUITRY APPROACHES TO UNDERSTANDING THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA

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PAST AND PREVAILING PATHOPHYSIOLOGIC MODELS OF PSYCHIATRIC DISORDERS

Models of the nature of brain dysfunction in major psychiatric disorders have evolved substantially over the past several decades in parallel with the progression in knowledge resulting from investigations of the neurobiological mechanisms that contribute to normal cognition, emotion, and behavior. Some models of psychopathology have emphasized, in their simplest forms, the central contribution of excesses or deficits in the functional activity of a given neurotransmitter to the disease process of interest. In general, these models have been very useful in motivating investigations of the molecular underpinnings and biochemical functions of the neurotransmitter systems of interest, and in spurring the development of novel psychopharmacologic agents that influence these systems. However, in extreme cases, these models tended to view individual psychiatric disorders as the consequences *solely* of the postulated disturbance in the neurotransmitter of interest, and consequently they tended to be conceptually similar to historic views that altered levels of the four classical bodily humors produced different forms of madness. In addition to this limited conceptual perspective, neurotransmitter-based models sometimes seemed to attribute behavioral, emotional, or cognitive functions to neurotransmitters, instead of explicitly recognizing that neurotransmitters have defined actions on receptors, whereas behaviors, emotions, and cognitive abilities represent emergent properties of the integrated activity of large networks of neurons.

Other models of psychopathology have emphasized the critical role of localized disturbances in individual brain regions, an approach that, in extreme cases, has been critiqued as ''neophrenology.'' Although these models have been useful in stimulating studies of the structure–function relationships of the implicated brain regions, they have been limited in a number of respects, including the inability to account for the array of signs and symptoms that typically constitute the syndromic diagnosis of a psychiatric disorder.

These two types of models were influenced, at least in part, by extrapolations from earlier successes in the study of Parkinson's disease, which was then viewed as a single neurotransmitter (e.g., dopamine) disease owing to a localized neuropathology (e.g., cell death in the substantia nigra). However, in recent years, these two general approaches have given way to neural circuitry-based models that reflect a fuller appreciation of the fact that neurotransmitters act in an anatomically constrained fashion to produce specific biochemical effects at the cellular level, and that the localization of function(s) is a consequence of the flow of information processing through the neural circuits within a given brain region and those linking that region to other brain areas. These latter types of models (1–3) incorporate the recognition that complex brain functions, such as those that are disturbed in major psychiatric disorders, are subserved by the coordinated activity of distributed ensembles of neurons.

Consequently, the goal of this chapter is to examine the use of a neural circuitry-based approach to understanding the pathophysiology of a psychiatric disorder, using studies of the neurobiological basis for cognitive dysfunction in schizophrenia as an example. Specifically, this chapter: (a) considers the convergent lines of evidence that suggest that the neural circuitry involving the dorsal prefrontal cortex is disturbed in this disorder, (b) reviews the normal organization of this circuitry as revealed through studies in animals, (c) assesses the evidence regarding the integrity of this circuitry in schizophrenia, and (d) discusses new opportunities

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for neural circuitry-based studies of the pathophysiology of schizophrenia.

EXAMINING SCHIZOPHRENIA AS A DISORDER OF NEURAL CIRCUITRY

Clues from the Clinical Syndrome

Although schizophrenia and Alzheimer's disease (AD) both received these appellations approximately a century ago, their initial descriptions were based on different types of data. For schizophrenia, the disorder was recognized by the presence of a constellation of clinical features and a particular longitudinal course, whereas the identification of AD was based on clinicopathological correlations. Indeed, the observation of neurofibrillary tangles and neuritic plaques in the cerebral cortex of the victims of AD provided the foundation for the rich array of anatomic, biochemical, and molecular genetic studies in the past two decades that have produced the wealth of current knowledge regarding the pathophysiology of this disease.

In contrast, studies of the pathophysiology of schizophrenia continue to depend, in large part, on clues derived from the clinical syndrome. Although the number and diversity of the clinical features of this illness make this approach daunting, a reasonable case can be made that the disturbances in cognition commonly seen in schizophrenia represent a core feature of the illness. For example, at least some of the other signs and symptoms of schizophrenia may be conceptualized as secondary to the cognitive disturbances (4), cognitive abnormalities can be identified during the prodromal phase of the illness and in those at increased risk for the disorder (5), cognitive symptoms appear to be persistent across the course of the illness (6), and the severity of cognitive impairment may be the best predictor of long-term outcome (7,8). Thus, the clinical features of schizophrenia argue for an emphasis on the neural circuitry that normally subserves the types of cognitive functions that are disturbed in the disorder.

Clues from Developmental Features

Although the idea that schizophrenia is a late consequence of an early developmental lesion (9) has many merits, direct evidence for a brain abnormality that could be explained by a mechanism operating prior to or at the onset of the clinical symptoms of the illness, and that could not be attributed to factors associated with having the illness, has proven difficult to obtain. For example, reports of cytoarchitectural disturbances in the entorhinal cortex of schizophrenic subjects (10,11) attracted a great deal of attention because the reported findings were strongly suggestive of an abnormality in neuronal migration (12); however, subsequent studies with larger sample sizes have both failed to confirm these reports and have provided likely methodologic explanations for the initial findings (13–16). The report of an altered distribution of interstitial neurons in the subcortical white matter was also conceptually very attractive because it strongly suggested an early developmental lesion (17); however, the majority of schizophrenic subjects appear to lack such abnormalities (18,19).

However, one of the characteristics of schizophrenia is the tendency for clinical symptoms to first appear during late adolescence or early adulthood, and it has been argued that hypotheses of the pathophysiology of schizophrenia must accommodate this age of onset (9,20). Although the average age of first hospitalization for patients with schizophrenia is in the early or mid-twenties for men and women, respectively (21,22), psychotic symptoms may appear months or even years prior to hospitalization (22–24). In addition, deterioration in other areas, such as scholastic performance and sociability, precedes the onset of the overt symptoms of schizophrenia by some time (5,23,24), and may represent strong predictors of the subsequent appearance of the disorder. Thus, developmental events occurring during the second decade of life may play a critical role in the appearance of cognitive dysfunction in schizophrenia.

Clues from Distributed Brain Abnormalities

Structural and functional neuroimaging studies in subjects with schizophrenia have implicated a number of brain regions as potential sites of dysfunction or morphologic abnormalities. For example, certain brain regions, such as the medial temporal lobe (including the hippocampus, amygdala, and parahippocampal gyrus), the superior temporal gyrus, the dorsal prefrontal cortex and the thalamus, have all been shown to have reduced total volume in subjects with schizophrenia, although the magnitude of the decrease and its consistency across studies has not been uniform (25). Similarly, functional imaging studies have shown alterations in the activation of some of these brain areas under different conditions, especially when subjects are performing tasks that normally are associated with a change in activation (26). Although some of the reported findings cannot be accommodated in this way, a number of the affected regions share reciprocal connections. Thus, pathophysiologic models that account for the reported abnormalities in two or more of these regions, and the connections that link them, may be particularly promising.

Incorporating These Types of Clues into Research Strategies for Identifying Neural Circuitry Abnormalities

Based on the three types of clues summarized in the preceding, one can ask whether they converge or triangulate on neural circuit(s) that may be preferentially involved in the pathophysiology of schizophrenia. Although this approach

suggests a number of possible candidate circuits for investigation, the dorsal prefrontal cortex (dPFC) may be considered a prototypic nodal point for circuit analysis in schizophrenia for the following reasons.

First, from the perspective of clinical clues, subjects with schizophrenia perform poorly on cognitive tasks that involve working memory, the ability to transiently maintain information in order to guide a subsequent response (27). For example, individuals with schizophrenia exhibit impairments on oculomotor delayed-response tasks (28), a cognitive paradigm on which nonhuman primates with structural or reversible cooling lesions of the dPFC perform poorly (29). Consistent with these observations, subjects with schizophrenia also fail to show normal activation of the dPFC when attempting to perform tasks that tap working memory (30).

Second, from the developmental perspective, the circuitry of the primate dPFC clearly undergoes marked refinements during adolescence, although certainly some other brain regions that have not been as well studied are also likely to show such changes. For example, the number of excitatory synapses in the dPFC declines by 50% during adolescence in both monkeys and humans (31,32). In addition, substantial changes occur in markers of excitatory, inhibitory, and modulatory inputs to pyramidal neurons in deep layer 3 of primate dPFC. The apparent laminar specificity of at least some of these changes raises the possibility that circuits involving these pyramidal cells may be preferentially affected in schizophrenia (33).

Third, from the perspective of regional brain analyses, the PFC has been shown to have subtle reductions in gray matter volume in a number of structural imaging studies of schizophrenia (25). The failure of other studies to detect such structural abnormalities in the PFC has been hypothesized to be a consequence of several factors, including a reduction in total PFC volume that approximates the level of sensitivity of MRI and the restriction of volumetric changes to certain regions or gyri of the dPFC (25). Consistent with these interpretations, postmortem observations indicate that cortical thickness in the dPFC may be reduced by 3% to 12% in subjects with schizophrenia (34–37), although these changes do not always achieve statistical significance. In addition, some (38–40), but not all (41), *in vivo* proton spectroscopy studies indicate that N-acetyl aspartate (NAA), a putative marker of neuronal and/or axonal integrity, is reduced in this brain region. Interestingly, the magnitude of these NAA changes in the dPFC was correlated with the degree of impaired activation in other brain regions during working memory tasks, raising the possibility that a neuronal abnormality in the dPFC could account for distributed functional disturbances in the working memory network (40).

Other lines of evidence suggest that these changes may reflect disturbances in the synaptic connectivity of the dPFC in schizophrenia. For example, the presence of decreased phosphomonoesters and increased phosphodiesters (42,43), as measured by P^{31} spectroscopy, in the PFC of schizophrenic subjects has been interpreted to reflect an increased breakdown of membrane phospholipids, and consequently a decreased number of synapses. In addition, a recent gene expression profiling study using cDNA microarrays found that the group of genes encoding proteins that regulate presynaptic secretory machinery were most consistently altered (44). Furthermore, reduced levels of synaptophysin, an integral membrane protein of small synaptic vesicles, have also been observed in the dPFC of subjects with schizophrenia in most (45–49), but not all (50), studies.

For these reasons, the next two sections of this chapter focus on a summary of the normal organization of dPFC circuitry and on a review of the evidence suggesting that this circuitry is disturbed in schizophrenia.

OVERVIEW OF DPFC CIRCUITRY IN PRIMATES

Direct studies of the circuitry of the human dPFC have obvious limitations; however, the available cross-species studies indicate that the macaque monkey brain provides an accurate and useful model system for understanding the general organization of the human dPFC. Thus, this section reviews the constituent cell types and the patterns of intrinsic and extrinsic connectivity that characterize the primate dPFC.

Cell Types

Pyramidal Neurons

About 70% of all cortical neurons are pyramidal cells (51). The majority of pyramidal cells have a characteristically shaped cell body that gives rise to a single apical dendrite that is oriented perpendicular to the cortical surface and frequently ends in a terminal tuft (Fig. 53.1). An array of shorter basilar dendrites spread in a radial fashion at the base of the cell body. Both the apical and basilar dendrites are coated with short protrusions or spines, which represent the principal targets of most excitatory synaptic inputs to pyramidal neurons. Because dendritic spines are actively formed or resorbed in response to changes in presynaptic inputs, dendritic spines provide a good estimate of the number of excitatory synapses that pyramidal cells receive (52). Typically, pyramidal neurons possess 6,000 to 10,000 dendritic spines (53). In addition to receiving one excitatory input, some dendritic spines also receive a synapse with the features suggestive of an inhibitory input. Inhibitory terminals also synapse on the dendritic shafts, cell body, and axon initial segment of pyramidal cells. Typically, pyramidal cells receive about 2,000 inhibitory synapses on dendritic shafts, 200 on the cell soma, and 20 on the axon initial segment (53).

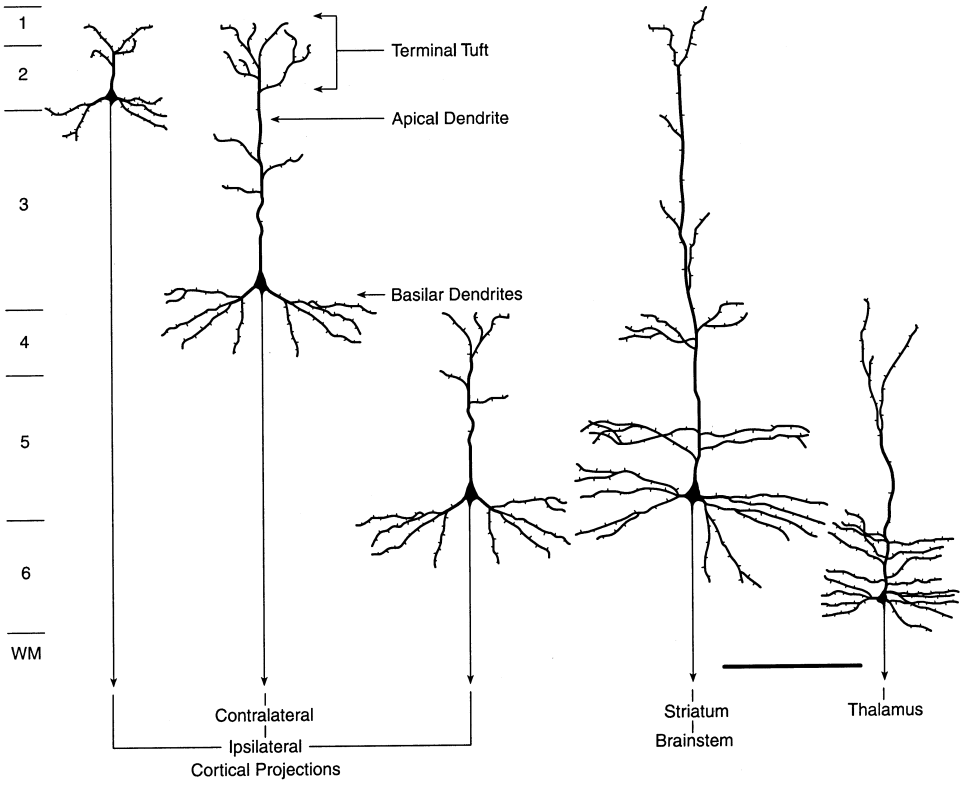

FIGURE 53.1. Schematic drawing illustrating the characteristic morphologic features of pyramidal neurons in different cortical layers. Note that the laminar location of the cell soma tends to be associated with the major projection target of the principal axon. Arabic numbers, cortical layers; WM, white matter. Adapted from Jones EG. Laminar distribution of cortical efferent cells. In: Peters A, Jones EG, eds. *Cerebral cortex,* vol 1. New York: Plenum Press, 1984:521–553.

The axons of pyramidal cells typically give rise to intrinsic collaterals, which travel either horizontally or vertically within the gray matter, and a principal axonal projection, which enters the white matter and travels to another brain region. These axons utilize excitatory amino acids, such as glutamate, as a neurotransmitter and form synapses that have the characteristic morphology associated with excitatory neurotransmission. These so-called Gray's Type I synapses are characterized by the presence of small round vesicles in the axon terminal, and a postsynaptic density that is thick and asymmetric in appearance (54).

Nonpyramidal Neurons

Of the other major type of cortical neurons, nonpyramidal cells, about 90% utilize the inhibitory neurotransmitter γ-aminobutyric acid (GABA). The axons of these generally small, local circuit or interneurons, arborize within the cortical gray matter and form Gray's Type II synapses that are characterized by pleomorphic vesicles in the axon terminal and symmetric pre- and postsynaptic densities. GABA neurons constitute approximately 25% of all neurons in the primate neocortex (55), and are comprised of about 12 distinct subtypes (56,57). Although the differences among subtypes can be described on the basis of the morphologic features of the cell body and proximal dendrites (e.g., bipolar, multipolar, bitufted), the most discriminating and functionally meaningful classification system is based on the organization of the axonal arbor and synaptic targets of the axon terminals. In addition, GABA neurons are chemically heterogeneous, and separate subpopulations can be identified by the presence of specific neuropeptides or calcium-binding proteins (58,59).

Together, these morphologic and chemical features define subpopulations of GABA neurons that appear to have different biophysical properties and different roles in dPFC circuitry (Fig. 53.2). For example, GABA neurons of the chandelier class, which may also express either the neuropeptide corticotropin-releasing factor (60) or the calcium-binding protein parvalbumin (61,62), are found primarily in cortical layers 2 to 5 in monkey dPFC (56). The axon terminals of these neurons, which are arrayed as distinct vertical structures (termed "cartridges"), form Gray's Type II synapses exclusively with the axon initial segment of py-

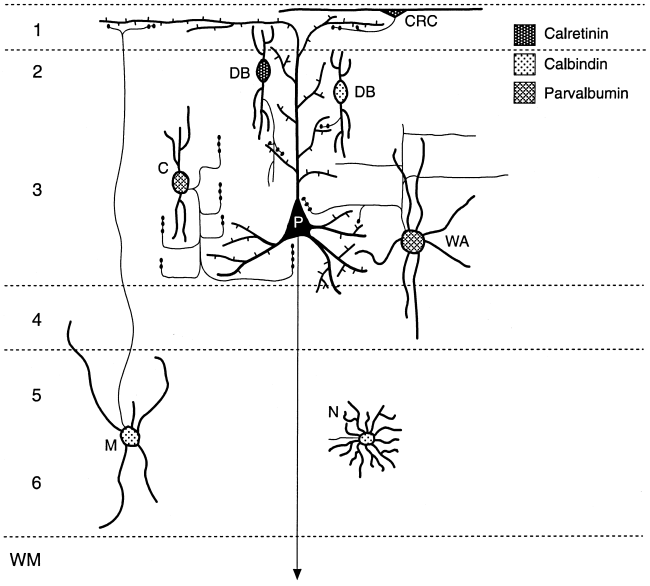

FIGURE 53.2. Schematic drawing of the synaptic interactions between different classes of local circuit neurons and a layer 3 pyramidal neuron (P) in monkey prefrontal cortex. C, parvalbumin (PV)-labeled chandelier neuron; CRC, calretinin (CR)- and/or CB (calbindin)-labeled Cajal-Retzius neuron; DB, CR- or CB-labeled double-bouquet neuron; M, CB-labeled Martinotti cell; N, CB-labeled neurogliaform neuron; WA, PV-labeled wide arbor (basket) neuron. Modified from Condé F, Lund JS, Jacobowitz DM, et al. Local circuit neurons immunoreactive for calretinin, calbindin D-28k, or parvalbumin in monkey prefrontal cortex: distribution and morphology. *J Comp Neurol* 1994;1:95–116.

ramidal neurons (63–68), the site of action potential generation in pyramidal cells. Each chandelier cell may contact up to 300 pyramidal neurons within a radius of 100 to 150 μm from its cell body (69). Thus, chandelier cells exert critical inhibitory control over the activity of a localized group of pyramidal neurons. In contrast, the axons of wide arbor (basket) neurons spread horizontally for considerable distances (up to 1.0 mm) within the dPFC (56) and form Gray's Type II synapses with the cell bodies, dendritic shafts, and dendritic spines of pyramidal neurons (70). Wide arbor neurons may be specialized to provide inhibitory constraints over the activity of spatially segregated populations of PFC pyramidal neurons (71,72). A third example, double bouquet cells, which contain the calcium-binding proteins calbindin or calretinin (58), have radially restricted axonal arbors that synapse with the dendritic shafts of both pyramidal and local circuit neurons (73).

Laminar Arrangement of Neurons

The dPFC, like other cortical association regions, is composed of six layers that can be distinguished according to the size and packing density of their constituent neurons (Fig. 53.3). Layer 1, which is located just below the pial surface, contains relatively few neurons, but approximately 90% of these neurons utilize GABA. Layers 2 and 4 are thin and densely packed with small "granular" cells. The majority of these neurons are small pyramidal cells, and GABA neurons constitute approximately 30% and 15% of all neurons in layers 2 and 4, respectively (55). Layers 3 and 5, the thickest cortical layers, contain prominent pyramidal neurons with a classic morphology. In both of these layers, the size and packing density of the pyramidal neurons is greater near their borders with layer 4. These patterns make it possible to subdivide layers 3 and 5 into superficial (toward the pial surface) and deep zones. Pyramidal cells in layer 6 have a modified or atypical appearance. In general, GABA cells constitute 20% to 30% of the neurons in layer 3, and about 15% of the neurons in layers 5 and 6 (55).

Different types of axonal projections to the dPFC terminate in certain cortical layers, and projections from the dPFC to other brain regions generally originate from pyramidal neurons in specific lamina. Thus, the laminar specificity of abnormalities in the dPFC in schizophrenia may reveal information about the types of connections that are affected. Nevertheless, owing to the vertical spread of the dendrites of many neurons, alterations in afferents to one layer can influence the function of neurons whose cell body is located in a different layer.

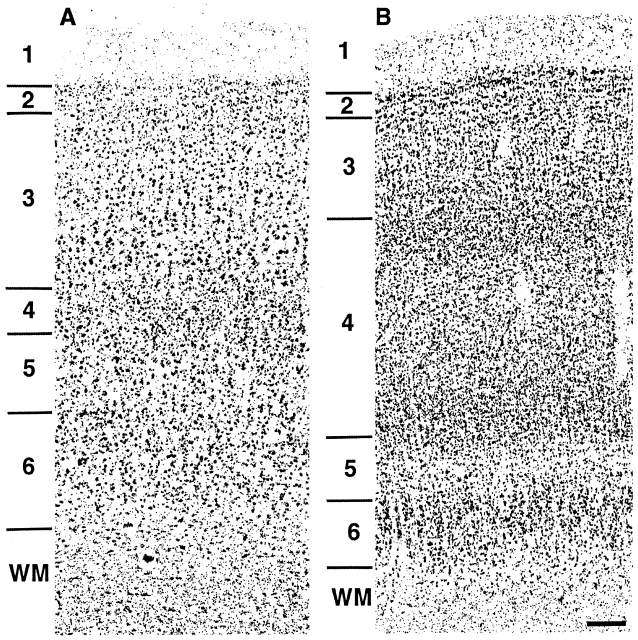

FIGURE 53.3. Nissl-stained sections showing laminar and regional differences in cell size in packing density in area 46 *(A)* of the human prefrontal cortex and area 17, primary visual cortex *(B)*. Calibration bar = 200 μm. Modified from Lewis DA. The organization of cortical circuitry. In: Harrison PJ, Roberts GW, eds. *The neuropathology of schizophrenia: progress and interpretation.* Oxford: Oxford University Press, 2000:235–256.

Organization of Projections from the dPFC

Pyramidal neurons located in layers 2 and superficial 3 tend to project to nearby cortical regions in the same hemisphere, whereas those in deep layer 3 frequently project to more distant ipsilateral regions or across the corpus callosum to cortical regions in the other hemisphere (Fig. 53.1). Projections within the same hemisphere are termed associational projections, whereas those to the contralateral hemisphere are termed callosal projections. Pyramidal cells in layers 5 and 6 project subcortically, with outputs from layer 5 directed to the striatum, superior colliculus, and other subcortical structures, and those from layer 6 preferentially directed to the thalamus. The efferent axons of pyramidal neurons tend to project to only one other brain region, although a small percentage of these neurons do give rise to collateral projections (74,75). In contrast to these extrinsic projections, the small pyramidal cells in layer 4 project primarily within the gray matter and relatively few send axons into the underlying white matter.

Although this laminar distribution of cortical efferents is generally accurate, many exceptions exist. For example, 25% to 30% of the pyramidal neurons furnishing associational projections to other PFC regions are located in the infragranular layers (layers 5 and 6) (74), and approximately 15% to 20% of neurons projecting to the striatum are located in layer 3 (76). In addition, the nature of the cortical output to a given region may vary with the location of the cell body of origin. For example, neurons in layer 6 provide "modulatory" inputs to cells in higher order thalamic nuclei (such as the mediodorsal thalamic nucleus, the principal source of thalamic projections to the dPFC) as well as inputs to the thalamic reticular nucleus, which regulates thalamocortical interactions. In contrast, thalamic projections originating in layer 5 do not innervate the reticular nucleus and appear to provide "driving" afferent inputs to higher order nuclei (77).

The innervation patterns of the intrinsic axon collaterals of pyramidal cells also tend to differ across cortical layers (71). Pyramidal neurons in layers 2 and 3 furnish local collaterals that arborize in the vicinity of the cell body, as well as horizontal axon projections that spread for considerable distances through the gray matter and then give rise to discrete clusters of axon terminals in the supragranular layers. Although pyramidal neurons in layers 5 and 6 also furnish horizontal intrinsic collaterals, these have a more limited spread and do not terminate in spatially segregated clusters. In contrast, pyramidal cells in layer 4 furnish predominantly vertically oriented axons, which appear to be specialized for interlaminar connections. The intrinsic axonal connections of pyramidal neurons in layers 2 and 3 of the dPFC also appear to be specialized relative to the homologous neurons in at least some other cortical regions. For example, although approximately 95% of the long distance intrinsic and associational axon projections of these neurons target the dendritic spines of other pyramidal cells (78), the synaptic targets of the local axon collaterals of these cells are equally divided between spines and dendritic shafts of GABA neurons (79).

Organization of Projections to the dPFC

The dPFC shares connections with a number of other cortical regions (Fig. 53.4). The inputs from these areas frequently terminate across all cortical layers, although the different layers tend to be preferentially innervated depending upon the source of the inputs. For example, inputs from cortical regions that have a well-developed layer 4 tend to terminate more prominently in layers 4 to 6, whereas those that originate in regions with a poorly developed layer 4 tend to terminate in layers 1 to 3 (80). In some cases, afferents from different regions (e.g., callosal inputs from the contralateral PFC and associational inputs from the posterior parietal cortex) tend be distributed into interdigitated columns (81).

In contrast to these cortical inputs, afferents from thalamic relay nuclei, such as the medial dorsal nucleus, project dominantly to layers deep 3 and 4, with a minor projection to layer 6 (82). Although afferents from the amygdala project more densely to orbital than dorsal regions of the PFC, these tend to terminate in layers 1 and 6 (83).

Subcortical nuclei containing monoamines or acetylcholine also exhibit distinct laminar patterns of termination in the PFC, along with substantial regional differences in rela-

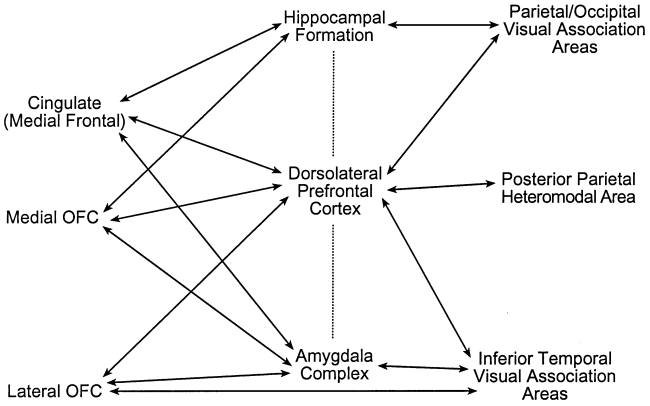

FIGURE 53.4. Schematic diagram of principal corticocortical connections within the frontal lobe regions, including primary limbic connections, and between posterior sensory cortices and frontal lobe regions. Double-head arrows indicate that most connections are reciprocal. Relatively sparse direct connections between dorsolateral prefrontal cortex and limbic structures (hippocampal formation and amygdala nuclei) are depicted with a dashed line. OFC, orbitofrontal cortex. Modified from Kaufer D, Lewis DA. Frontal lobe anatomy and cortical connectivity. In: Miller BL, Cummings JL, eds. *The human frontal lobes: functions and disorders.* New York: Guilford Publications, 1998:27–44.

tive density. Dopamine (DA)-containing axons from the ventral mesencephalon have a bilaminar distribution in the PFC (84), forming a dense band in layers 1 through the most superficial portion of layer 3 and a second band of lower density in layers deep 5 and 6. In more densely innervated regions, such as dorsomedial PFC (area 9), labeled axons are also present in high density in the middle cortical layers, forming a third distinctive band in deep layer 3. The noradrenergic (NA) projection from the locus coeruleus exhibits a different, and in some ways complementary, laminar innervation pattern to that of DA axons (85,86). The density of NA axons is substantially greater in the deep cortical layers, especially layer 5, than in the more superficial cortical laminae. In particular, few NA axons are present in layer 1, which receives a dense DA innervation. In contrast, the relatively uniform laminar distribution of cholinergic (87) and serotonergic (88) axons contrasts with the substantial heterogeneity exhibited by both DA and NA axons.

INTEGRITY OF PREFRONTAL CORTICAL CIRCUITRY IN SCHIZOPHRENIA

In this section, we consider how this knowledge about the normal organization of dPFC circuitry can be used to interpret studies of the integrity of different types of neural elements in subjects with schizophrenia. As noted, several lines of evidence support the hypothesis that schizophrenia is associated with a decrease in the synaptic connectivity of the dPFC. However, these abnormalities do not appear to be a consequence of a decreased complement of dPFC neurons, because several postmortem studies (34,37,89) have reported either a normal or increased cell packing density in the dPFC. In addition, the one study that used unbiased approaches to determine the total number of PFC neurons did not observe a reduction in subjects with schizophrenia (90); however, the approaches used in these studies probably lacked adequate sensitivity to detect reduced numbers of small subpopulations of PFC neurons. Some studies that focused on certain neuronal subpopulations have reported decreases in density of small neurons in layer 2 (91) or of the parvalbumin-containing subpopulation of GABA neurons (92). However, the latter abnormality was not observed in another study (35), and it should be noted that a reduction in neuronal density when using immunocytochemical markers might reflect an alteration in the target protein rather than in the number of cells.

Smaller neuronal cell bodies could also contribute to the observed reduction in PFC gray matter in schizophrenia. Interestingly, two groups have reported that the somal volume of pyramidal cells in deep layer 3 of dPFC area 9 is decreased in subjects with schizophrenia (89,93). In addition, this reduction in somal volume may be associated with a decrease in total length of the basilar dendrites of these neurons (94). In contrast, the size of GABA neurons does not appear to be reduced (35,95), although less rigorous methods were employed in these studies.

In summary, although a reduction in neuron number cannot be completely excluded, the subtle reduction in dPFC gray matter in schizophrenia may be attributable to a combination of smaller neurons and a decrease in dPFC neuropil, the axon terminals, distal dendrites and dendritic spines that represent the principal components of cortical synapses. Indeed, as described in more detail below, these two factors may be interrelated.

Candidate Sources for Synaptic Reductions

The apparent reduction in synaptic connectivity in the dPFC of subjects with schizophrenia may be attributable to one or more of the following sources of synapses: axon terminals intrinsic to the dPFC, or afferents from other cortical regions, the thalamus, or other subcortical locations. Although none of these sources can be excluded at present, some are more likely to be major contributors than others. For example, one subcortical source, the DA projections from the mesencephalon, may be reduced in number in schizophrenia as evidenced by the report of diminished densities of axons immunoreactive for tyrosine hydroxylase, the rate limiting enzyme in catecholamine synthesis, and the DA membrane transporter in the dPFC (96). However, these reductions appeared to be restricted to the deep cortical layers. Furthermore, an *in vivo* neuroimaging study found a reduced density of DA D1 receptors in the dPFC of subjects with schizophrenia (97); however, DA axons are estimated to contribute less than 1% of cortical synapses (84). Consequently, even the complete loss of DA projections to the dPFC could not, in isolation, account for the observed reductions in gray matter volume or synaptophysin protein levels. The relatively small contributions that NA-, serotonin-, and acetylcholine-containing axons make to the total number of synapses in the dPFC also argues against disturbances in these systems as the principal cause of reduced synaptic connectivity in this brain region.

The fact that layer 3 pyramidal cells, which give rise to a substantial number of intrinsic excitatory synapses, have reduced somal volume in subjects with schizophrenia (89, 93) may suggest that the synapses furnished by the intrinsic axon collaterals of these neurons are reduced in number, because somal volume tends to be correlated with the size of a neuron's axonal arbor (98,99). Evidence for a disturbance in intrinsic connectivity is supported by recent studies using cDNA microarray profiling of the expression of over 7,000 genes in dPFC area 9 of subjects with schizophrenia (44). Among 250 functional gene groups, the most marked changes in expression were present in the group of genes that encode for proteins involved in the regulation of presynaptic neurotransmitter release. Although these findings very likely indicate a general impairment in the efficacy of synaptic

transmission within the dPFC in schizophrenia, whether they represent a "primary" abnormality intrinsic to the dPFC or a "secondary" response to altered afferent drive to this brain region remains to be determined. Furthermore, because the specific genes in this group that were most altered appeared to differ across subjects, it seems unlikely that these findings can be explained solely by reduction in the number of intrinsic dPFC synapses. Consistent with this view, the expression of synaptophysin mRNA does not appear to be reduced in the dPFC of subjects with schizophrenia (50,100), suggesting that the reduction in this synaptic protein marker in the dPFC may have an extrinsic source. Consistent with this interpretation, synaptophysin mRNA levels are reduced in cortical areas that do furnish projections to the dPFC (101,102). However, whether these transcriptional changes are present in PFC-projecting neurons, and if so, whether they result in reduced levels of synaptophysin protein in the terminal fields of these neurons, have not been assessed.

Decreased synaptic connectivity within the PFC might also be attributable to altered inputs from the thalamus. For example, some structural MRI studies have revealed a reduction in thalamic volume in subjects with schizophrenia (103–106). In addition, thalamic volume was correlated with prefrontal white matter volume in schizophrenic subjects (107), suggesting that a reduction in thalamic volume was associated with fewer axonal projections to the PFC. Consistent with these observations, postmortem studies have revealed reductions of 17% to 25% in volume and 27% to 40% in total neuron number of the medial dorsal thalamic nucleus (MDN), the principal source of thalamic projections to the PFC (108–110). Interestingly, the available data suggest that these abnormalities exhibit topographic specificity. For example, reduced cell numbers have been reported in the MDN and the anterior thalamic nuclei (which project to the PFC and anterior cingulate cortex), whereas the ventral posterior medial nucleus, a sensory relay nucleus, appears to be unaffected (109,110). In addition, within the MDN, one study indicates that neuron number is significantly decreased in the parvocellular subdivision (which projects principally to the dPFC), but not in the magnocellular subdivision (which projects principally to the ventral PFC) (110). Finally, studies in both subjects with schizophrenia who never received antipsychotic medications (111) and monkeys treated for 1 year with haloperidol (112) suggest that these medications do not account for the reduction in MDN neuron number. However, despite the apparent consistency of the published studies, a deficient number of MDN neurons in schizophrenia must still be considered a preliminary finding given the relatively small sample sizes reported to date, and the fact that potential confounds, such as comorbid conditions (e.g., alcoholism), have not been adequately assessed.

Certainly, a reduction in cell number in the MDN could contribute both to the decrease in synaptic markers in the PFC, and, given the dependence of working memory tasks on the integrity of thalamo–prefrontal connections (29), to the disturbances in working memory observed in schizophrenia. However, in contrast to other species, the primate MDN contains both cortically projecting neurons and local circuit neurons. Thus, it is critical to determine which subpopulation(s) of MDN neurons are affected in schizophrenia. Interestingly, the density of neurons in the anterior thalamic nuclei that contain parvalbumin (113), a calcium-binding protein present in thalamic projection neurons (114), is reduced in schizophrenia; however, whether this reduction represents an actual loss of neurons, as opposed to an activity-dependent decrease in parvalbumin expression, is not known.

Within the dPFC, five other lines of evidence are also consistent with a reduction in inputs from the MDN (Fig. 53.5). First, a preliminary report notes that subjects with schizophrenia, but not those with major depression, have a decreased density of parvalbumin-labeled varicosities (putative axon terminals) in layers deep 3 and 4, the principal termination zone of thalamic projections to the PFC (115). In contrast, parvalbumin-labeled varicosities were not decreased in layers 2 to superficial 3, suggesting that the reduction in layers deep 3 and 4 might not be attributable to changes in the axon terminals of the parvalbumin-containing subset of cortical GABA neurons present in cortical layers 2 to 5 (35). Thus, the laminar-specific reduction of

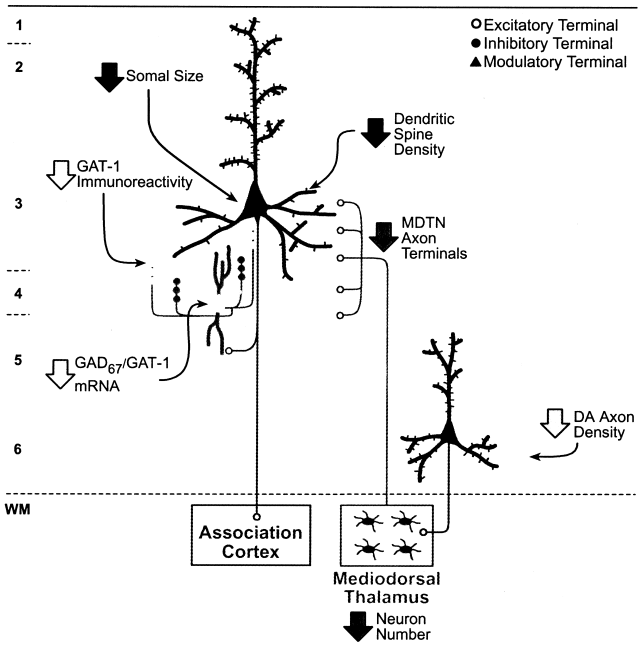

FIGURE 53.5. Schematic diagram summarizing disturbances in the connectivity between the mediodorsal (MD) thalamic nucleus and the dorsal prefrontal cortex (PFC) in schizophrenia. See text for details. Modified from Lewis DA, Lieberman JA. Catching up on schizophrenia: natural history and neurobiology. *Neuron* 2000;28:325–334.

parvalbumin varicosities in schizophrenia is consistent with a decreased number of MDN terminals in the dPFC, although a laminar-specific reduction in the axon terminals of local circuit neurons cannot be conclusively excluded.

Second, the thalamic projections to the dPFC principally target the dendritic spines of pyramidal neurons (116). In experimental animals, the elimination of presynaptic axon terminals leads to a resorption of the postsynaptic dendritic spine (117), suggesting that a reduction in MDN projection neurons would be associated with decreased dendritic spine density in the dPFC. The two studies that have examined this issue both found decreased spine density on the basilar dendrites of PFC layer 3 pyramidal neurons (94,118), with this decrease most marked for pyramidal neurons located in deep layer 3 (94), those most likely to be targeted by projections from the thalamus. Although the well-documented, remarkable plasticity of dendritic spines must be considered when interpreting these findings, these observations are consistent with a reduction in MDN–dPFC connectivity in schizophrenia. However, the presence of more modest reductions in spine density on pyramidal neurons in cortical layers that do not directly receive MDN input suggest that the observed decrease in deep layer 3 may reflect the combined effect of a deficient number of thalamic and cortical synapses (94).

Third, the size of layer 3 pyramidal neurons is reduced in the dPFC of schizophrenic subjects (89,119). Although the possible relationship of these findings to a decrease in MDN inputs is less clear, studies in animals have provided evidence of denervation atrophy of layer 3 pyramidal cells following the loss of other afferent inputs (120).

Fourth, in the primate visual system, monocular deprivation, which results in reduced afferent drive from the thalamus, is associated with a decline in markers of activity in cortical GABA neurons (121), including decreased expression of the mRNA for glutamic acid decarboxylase (GAD_{67}), the synthesizing enzyme for GABA (122). Although the experimental manipulation of the visual system did not involve a partial reduction in thalamic neuron number, if these findings in the visual cortex can be generalized to a deficient number of MDN projections to the dPFC, a reduction in GAD_{67} in the dPFC of schizophrenic subjects might be expected. Consistent with this prediction, both GAD_{67} mRNA and protein levels have been reported to be reduced in the dPFC of schizophrenic subjects (26,123, 124), observations supported by other evidence of reduced GABA neurotransmission in the PFC of schizophrenic subjects. (See ref. 125 for review.)

Finally, the reduction in GAD_{67} mRNA expression in schizophrenia appears to be restricted to a subpopulation of PFC GABA neurons (approximately 25% to 30%), especially those neurons located in the middle cortical layers (124). Consistent with this finding, other studies suggest that the affected subpopulation of GABA neurons includes chandelier cells. The axon terminals of chandelier cells form distinctive vertical arrays (termed cartridges) that synapse exclusively on the axon initial segment of pyramidal neurons (126). Interestingly, expression of the mRNA for GABA membrane transporter (GAT-1) is also undetectable in approximately 25% to 30% of GABA neurons, which have a laminar distribution similar to the neurons with undetectable GAD_{67} mRNA expression (127). In addition, the density of GAT-1 immunoreactive chandelier neuron axon cartridges is decreased in the dPFC of schizophrenic subjects, with the reduction most evident in the middle cortical layers (128,129). Thus, given the powerful inhibitory control that chandelier neurons exert over pyramidal cell output, decreased excitatory thalamic drive to the PFC may be partially compensated for by a reduction in chandelier cell-mediated inhibition at the axon initial segment of layer 3 pyramidal cells. This effect could occur via the local axon collaterals of layer 3 pyramidal cells, approximately 50% of which target the dendritic shafts of GABA neurons (79). However, it is important to note that other causes and consequences of the observed alterations in chandelier neurons have not been excluded. (See ref. 124 for a discussion.)

Together, these data are all consistent with the hypothesis that schizophrenia is associated with abnormalities in the projection from the MDN to the dPFC. As in other cortical regions, the connections between the MDN and dPFC are reciprocal, which raises the question of whether the abnormal thalamocortical projection is paralleled by a disturbance in the corticothalamic projection. Studies that have examined PFC neurons in layers 5 and 6, the principal location of corticothalamic projection neurons, have generally not found evidence of a decrease in neuron size or number (34, 89,91), although one study (95) did report decreased neuronal density in layer 6 of PFC (region not specified); however, a reduced density of DA axons was observed selectively in layer 6 of dPFC area 9 in schizophrenic subjects (96). Interestingly, the dendritic shafts and spines of pyramidal cells are the principal synaptic targets of DA axon terminals in layer 6, and DA appears to play a critical role in regulating the influence of other inputs on pyramidal cell activity (130). Thus, a shift in DA neurotransmission in dPFC layer 6 could reflect a change in the modulation of corticothalamic feedback in response to abnormal thalamocortical drive (96).

The significance of these findings depends, in part, on the extent to which they are unique to the diagnosis of schizophrenia, and not a consequence of other factors associated with schizophrenia, such as antipsychotic drug treatment. Some of these findings have been examined for diagnostic specificity, whereas others have not. For example, the reduction in dendritic spine density on deep layer 3 pyramidal neurons was not found in subjects with major depressive disorder (94), and the reduction in density of GAT-1 immunoreactive axon cartridges was not apparent in subjects with nonschizophrenic psychiatric disorders (129). Similarly, to the extent to which it has been examined, these

findings do not appear to be attributable to the abuse of alcohol or other substances, which frequently accompanies the diagnosis of schizophrenia, although further studies in this area certainly are needed.

Similarly, the available evidence suggests that these disturbances in thalamo-prefrontal circuitry are not attributable to treatment with antipsychotic medications. Globally, the increase in cell packing density in the dPFC observed in subjects with schizophrenia was not found in monkeys treated for 6 months with a variety of antipsychotic agents (131). Similarly, treatment of monkeys for 12 months with haloperidol and benztropine at blood levels known to be therapeutic in humans was not associated with a reduction in the size or total neuron number of the MDN (112). The potential influence of antipsychotic drugs on GAT-1–labeled axon cartridges has been examined in several ways with interesting results (129). The density of labeled cartridges was greater in schizophrenic subjects who were on than off antipsychotic medications at the time of death (although both groups showed reduced levels compared to normal controls). In addition, compared to matched control animals, the density of GAT-1-positive cartridges was elevated in monkeys treated for 1 year with haloperidol. Thus, the convergence of these findings suggests both that the pathophysiology of schizophrenia may actually be associated with more marked reductions in GAT-1–immunoreactive cartridge density than those observed in postmortem studies.

OPPORTUNITIES FOR NEURAL CIRCUITRY-BASED STUDIES OF SCHIZOPHRENIA

The data summarized in the preceding section suggest that neural circuitry-based approaches to the study of brain abnormalities in schizophrenia provide: (a) a useful framework to account for the abnormalities observed in individual studies, (b) a platform for the formulation of predictions regarding the outcome of future studies, and (c) the promise of an enhanced ability to understand the neurobiological bases of clinical phenomena. However, a truly neural circuitry-based model of schizophrenia requires an appreciation of the mechanistic relations among the abnormalities observed in different components of the circuitry. Specifically, understanding the pathophysiology of schizophrenia (or any other psychiatric disorder) depends ultimately on knowing how abnormalities in one brain region or circuitry component produce and/or result from disturbances in others, a task that involves a consideration of cause, consequence, and compensation (132). Does a given abnormality represent a primary pathogenetic event (cause), does it reflect a downstream or upstream (given the reciprocal nature of many links between brain regions), secondary, deleterious event (consequence), or does it reveal a homeostatic response intended to restore normal brain function (compensation)? Distinguishing among these three possibilities for each component of a neural network will be necessary for understanding the pathophysiology of the disease as well as for developing novel therapies designed to correct causes and consequences and/or to augment compensatory responses.

Clearly, addressing these types of questions for schizophrenia requires several additional types of investigations. First, it is essential to further assess the normal organization of MDN–dPFC connectivity in nonhuman primates. For example, what patterns of connectivity within the dPFC link inputs from the MDN to the neurons that provide outputs to the MDN or to other brain regions such as the striatum? Second, MDN–dPFC circuitry needs to be further probed in subjects with schizophrenia in ways that inform an understanding of its functional integrity. For example, what are the postsynaptic consequences in pyramidal neurons of the apparent alterations in GABA neurotransmission in chandelier cells? Third, the direction of the pathophysiological changes in MDN–dPFC circuitry in schizophrenia need to be assessed in experimental animal models. For example, can the observations of altered spine density and decreased GAD_{67} mRNA expression in the dPFC be replicated by partial lesions of the MDN in monkeys? Do manipulations of neurotrophin expression in dPFC layer 3 pyramidal cells result in a loss of the MDN neurons that project to the dPFC?

It is also critical to extend these types of investigations to other brain regions that may integrate MDN–dPFC circuitry with broader neural networks. Besides the dPFC, the hippocampal formation is probably the brain region that has been most extensively studied in schizophrenia. Multiple imaging and postmortem studies have documented a slight bilateral reduction in the volume of the hippocampal formation (25,133), an observation supported by more recent *in vivo* proton spectroscopy findings of reduced hippocampal N-acetyl aspartate in both unmedicated adult and childhood onset subjects with schizophrenia (134). Although initial reports of hippocampal neuron disarray or misplaced neurons in the superficial layers of the adjacent entorhinal cortex have been widely cited, these observations have not been replicated in other studies. (See ref. 133 for review.) Reduced hippocampal volume also does not appear to be attributable to decreased neuronal number, but several independent studies have found reductions in neuronal cell body size in various subregions of the hippocampus proper (135–137). In addition, there are consistent reports of reductions in the gene products for synaptophysin and related presynaptic markers and in dendritic markers, such as microtubule-associated protein, in certain subdivisions of the hippocampus. (See ref. 12 for review.) Thus, these observations bear some similarity to findings in the dPFC in that disturbances in synaptic connectivity appear to be present in both regions in schizophrenia. How these findings inform our understanding of alterations in the intrinsic connectivity of the hippocampal formation in schizophrenia remains to

be determined, but their possible relation to dPFC abnormalities is suggested by experimental studies in rodents that indicate that dysfunction of the PFC appears postpubertally following perinatal lesions of the hippocampus (138).

As noted in the preceding section, it is also important to consider these findings within the context of the developmental time course of schizophrenia, especially the tendency for prodromal and clinical symptoms to become evident during the second and third decades of life. Although the adolescence-related pruning of excitatory (but not inhibitory) synapses (31,32) and their targets, pyramidal cell dendritic spines (139), in the primate dPFC has been well documented, little information exists regarding the extent to which different connections are actually affected by these changes. Interestingly, limited data suggest that the terminals of intrinsic axon collaterals from dPFC layer 3 pyramidal cells may be more extensively pruned than associational cortical projections to the dPFC (140). Knowing whether projections from the MDN are particularly vulnerable to this process might provide critical information for hypotheses regarding the mechanisms underlying disturbances in MDN–dPFC circuitry in schizophrenia.

Another current challenge to the types of neural circuitry-based models of schizophrenia illustrated herein is to understand how the genetic factors that confer susceptibility for the disease contribute to the observed alterations in neural circuitry. In other words, how can molecular genetic and systems neuroscience approaches be integrated in the study of schizophrenia? Schizophrenia appears to be a consequence of multiple interacting genes that individually may have relatively little effect; it is unlikely that all such genes are involved in every individual who meets diagnostic criteria for the disorder. Thus, assessment of the patterns of altered gene expression in the affected brain circuits of subjects with schizophrenia (using cDNA microarray technology or related techniques), and comparison of the chromosomal locations of these genes with regions implicated in schizophrenia through linkage studies (141), may provide convergent approaches to the identification of specific susceptibility genes. For example, as noted, a recent study of gene expression profiling in the dPFC of subjects with schizophrenia revealed that the group of genes encoding proteins involved in the regulation of presynaptic function were most consistently altered (44). In addition, although the subjects with schizophrenia appeared to share a common abnormality in the control of synaptic transmission, they differed in terms of the specific combination of genes that showed reduced expression, a finding that may be consistent with a polygenic model for this disorder. Interestingly, a number of the chromosomal loci that have been implicated in schizophrenia contain genes encoding proteins related to presynaptic function (44). However, this strategy, and the subsequent integration of potential susceptibility genes with neural circuitry models of the illness, rests on the prediction that genes regulating presynaptic function are not homogeneously expressed across classes of neurons and brain regions.

Finally, neural circuitry-based models must also be considered in relation to the clinical heterogeneity of schizophrenia. Is the magnitude of the abnormalities within MDN–dPFC circuitry related to the age of onset or severity of cognitive impairment? Can other clinical features be understood within the context of abnormalities in broader circuits that include connections with MDN and dPFC? Given the limitations involved in making rigorous clinicopathologic correlations in schizophrenia, the answers to these questions may await the development and application of *in vivo* imaging techniques with greater sensitivity, such as those that will permit functional assessments at the levels of individual cortical layers or thalamic nuclei.

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