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Unique Molecular Regulation of Higher-Order Prefrontal Cortical Circuits: Insights into the Neurobiology of Schizophrenia

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Abstract

Schizophrenia is associated with core deficits in cognitive abilities and impaired functioning of the newly evolved prefrontal association cortex (PFC). In particular, neuropathological studies of schizophrenia have found selective atrophy of the pyramidal cell microcircuits in deep layer III of the dorsolateral PFC (dlPFC) and compensatory weakening of related GABAergic interneurons. Studies in monkeys have shown that recurrent excitation in these layer III microcircuits generates the precisely patterned, persistent firing needed for working memory and abstract thought. Importantly, excitatory synapses on layer III spines are uniquely regulated at the molecular level in ways that may render them particularly vulnerable to genetic and/or environmental insults. Glutamate actions are remarkably dependent on cholinergic stimulation, and there are inherent mechanisms to rapidly weaken connectivity, e.g. during stress. In particular, feedforward cyclic adenosine monophosphate (cAMP)-calcium signaling rapidly weakens network connectivity and neuronal firing by opening nearby potassium channels. Many mechanisms that regulate this process are altered in schizophrenia and/or associated with genetic insults. Current data suggest that there are “dual hits” to layer III dlPFC circuits: initial insults to connectivity during the perinatal period due to genetic errors and/or inflammatory insults that predispose the cortex to atrophy, followed by a second wave of cortical loss during adolescence, e.g. driven by stress, at the descent into illness. The unique molecular regulation of layer III circuits may provide a nexus where inflammation disinhibits the neuronal response to stress. Understanding these mechanisms may help to illuminate dlPFC susceptibility in schizophrenia and provide insights for novel therapeutic targets.

Graphical abstract

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INTRODUCTION

Schizophrenia was originally termed “dementia praecox” (premature dementia) by Pick and Kraepelin due to the marked cognitive deficits that are a core feature of this illness.¹ Cognitive deficits in schizophrenia precede the onset of psychosis, continue throughout the lifetime of patients, and are the best predictor of long-term functional outcome.^{2,3} In particular, schizophrenia is characterized by profound alterations in the newly evolved prefrontal cortical (PFC) circuits that generate thought, manifesting in the clinical hallmarks of the disease.⁴ The PFC has extensive projections to provide top-down control of behavior, thought, and emotion (Figure 1A). Several lines of research indicate that the recurrent excitatory pyramidal cell microcircuits in deep layer III of the dorsolateral PFC (dlPFC) that generate thought are especially vulnerable in schizophrenia.⁵ These microcircuits are uniquely regulated at the molecular level in a manner that renders them susceptible to atrophy, as they contain the molecular machinery to magnify feedforward cyclic adenosine monophosphate (cAMP)-calcium signaling in spines, which may drive inflammation and weaken connections by opening nearby potassium channels. The molecular regulation of the dlPFC contrasts with “classical” sensory cortices (e.g., primary visual cortex), where cAMP–calcium signaling events strengthen connections,⁶ similar to those found in rodent sensory cortex. This review explores (1) how dlPFC circuits are regulated in a fundamentally different manner from classical circuits, (2) how genetic and environmental insults in schizophrenia may target these critical circuits, and (3) how strategies for protecting these synapses early in the course of illness may slow or lessen the progression of disease.

SCHIZOPHRENIA: BURDEN, ETIOLOGY, AND SYMPTOMS

Schizophrenia is a neurodevelopmental psychiatric disease that afflicts a large population (0.5–1%) globally.⁷ The various symptoms of schizophrenia patients can be categorized into three categories: (A) positive symptoms such as hallucinations and delusions that alter perceptions of reality and falsify normative behaviors, (B) negative symptoms that reflect the

absence of certain behaviors that are present in normal individuals such as flat affect, avolition, alogia, and anhedonia, and (C) cognitive symptoms such as thought disorder and working memory deficits.⁸ These symptoms usually manifest during late adolescence or early adulthood, with most patients experiencing a life-long course of the illness. Although these have been analyzed as distinct domains, the neurobiological underpinnings likely overlap, e.g. where symptoms such as alogia (poverty of speech), delusions, and hallucinations may all involve cognitive dysfunction of subsystems in the PFC.^{9,10} The disease is a highly heterogeneous syndrome, and the disease pathogenesis is multifactorial, involving several etiological factors that can aggravate the chance for developing the illness.¹¹ In general, it is thought that gene–environment interactions are pivotal in driving the pathogenesis of schizophrenia, with genetic predispositions and various environmental insults during prenatal, perinatal, and postnatal development enhancing risk for schizophrenia.^{12,13}

The “dual hit” hypothesis proposes that schizophrenia arises from initial insults during perinatal cortical formation, followed by a second wave of insults starting in adolescence that leads to onset of overt symptoms (Figure 2). Initial genetic and environmental insults would alter cortical formation in utero, afflicting cortical neuron migration and/or connectivity starting in the second trimester.¹⁴ These may include inherited or de novo genetic insults,^{15–19} including mosaic expression that may preferentially target the dlPFC and association cortices.^{20–22} A myriad of physiological stressors during pregnancy (e.g., maternal infection, immune dysregulation, maternal malnutrition, hypoxia) occurring at different stages of prenatal, perinatal, and postnatal development are associated with increased incidence of schizophrenia.^{12,23,24} The massively interconnected pyramidal cell circuits in layer III dlPFC may be especially vulnerable to insults that impact neuronal connectivity. The second age of vulnerability starts during adolescence (Figure 2). Recent neuroimaging data indicate an accelerated wave of cortical gray matter loss accompanies descent into illness,^{25,26} associated with increased signs of inflammation^{27,28} and often precipitated by stressful life events. Accelerated loss of gray matter may reflect exacerbations of the normal pruning process,^{29–32} although recent postmortem human data show the synaptic pruning in the PFC actually begins in childhood.³² Thus, additional, abnormal processes may be engaged during adolescence in schizophrenia. As stress exposure weakens PFC connectivity and function through increased catecholamine actions,^{33–35} and as there is increased dopamine (DA) innervation of layer III dlPFC in adolescence,^{36,37} stress exposure in adolescence may weaken already vulnerable dlPFC microcircuits.

COGNITIVE DEFICITS IN SCHIZOPHRENIA: LINKS TO THE DORSOLATERAL PFC

Cognitive deficits constitute a core feature of dysfunction in schizophrenia. They persist throughout the lifetime and are present in a majority of patients.^{1–3,38} Impairments in cognitive function can emerge prior to the onset of psychosis by almost a decade and are the best predictor of functional outcome.^{38–40} Impairments in cognitive function are present in medication-naïve, first-episode patients and also in a milder form in first-degree relatives of schizophrenia patients, suggesting that these deficits are not an artifact of neuroleptic

treatment and are intrinsic to the disease process.⁴¹ The degree of cognitive impairments also predicts the ability of individuals with schizophrenia to integrate into society and future employment potential.⁴² Although an active area of debate, researchers have recently called for schizophrenia to be categorized not as a psychotic disorder but rather a “cognitive illness”,¹ consistent with the original conceptualization of the illness as dementia praecox by Kraepelin in the Lehrbuch.

Imaging studies have a long history of identifying “hypofrontality” in the dlPFC of patients performing cognitive tasks that rely on the integrity of the PFC.^{43–45} These studies have also linked dlPFC cognitive dysfunction to symptoms of thought disorder and disorganization. For example, subjects with schizophrenia undergoing functional magnetic resonance imaging while performing the “n-back” sequential-letter working memory task, demonstrated hypofrontality of the dlPFC that strongly correlated with symptoms of thought disorder.⁴⁶ These data suggest that both working memory dysfunction and thought disorder involve perturbations to the dlPFC in schizophrenia patients. Neuroimaging studies in healthy volunteers have revealed an “inverted U” shaped relationship between dlPFC activation and working memory, exhibiting marginal levels of activity at low and high loads but significantly elevated activity at intermediate loads.⁴⁷ However, in schizophrenia, this inverted U appears to be left-shifted, indicating reduced activation at higher memory loads.⁴⁸ In spite of the fact that cognitive deficits in patients are central to outcome, existing pharmacotherapies are unable to ameliorate these debilitating symptoms. Therefore, understanding the neurobiology of the dlPFC and working memory provides necessary clues to understanding the etiology of the illness and strategies for normalizing cognitive operations.

MICROCIRCUIT RESPONSIBLE FOR WORKING MEMORY: ROLE OF THE DORSOLATERAL PREFRONTAL CORTEX

The dlPFC is essential for working memory. Lesions to the dlPFC produce profound and permanent deficits in working memory abilities.^{49,50} The dlPFC subserves our highest order functions, generating the mental representations that are the foundation for abstract thought and higher reasoning. The dlPFC creates a “mental sketch pad” through neural networks that can maintain information in the absence of external stimulation.⁵¹ Similar to humans, macaque monkeys display profound age-dependent improvements in working memory performance from infancy through adolescence and into adulthood that are characterized by sophisticated refinements in dlPFC circuitry that contribute to working memory maturation.^{52–54}

The primate PFC is topographically organized with extensive afferent and efferent projections with posterior cortex and subcortical regions^{55–57} (Figure 1A). The dlPFC has extensive reciprocal projections with the sensory and motor association cortices that are necessary for guiding thoughts, attention, and goal-directed actions.⁵⁵ The dlPFC has direct connections with the posterior hippocampus and also has widespread subcortical projections down to caudate, thalamus (mediodorsal and reticular nuclei), and to the cerebellar cortex via pons.^{58,59} In contrast, the medial PFC (mPFC) and orbital PFC represent and regulate

internal state and emotions by projecting to subcortical regions such as the amygdala, nucleus accumbens, and the hypothalamus.^{60–62} The PFC also can regulate our state of arousal through projections to the monoamine cell bodies located in the brain stem such as the locus coeruleus [source of norepinephrine (NE) projections], ventral tegmental area (VTA), and substantia nigra (SN) [the source of DA projections], and the dorsal raphe nucleus (DRN) to mediate serotonin/5-hydroxytryptamine (5-HT) cells.^{63–65} The orbital PFC also regulates cholinergic projections.⁶⁶ Thus, the PFC has widespread projections for “top-down” control of arousal state and of thought, action, and emotion.

The physiological role of the dlPFC has been extensively interrogated in monkeys performing working memory tasks (Figure 3). Pioneering studies revealed neurons in dlPFC that can maintain firing and represent information in the absence of sensory stimulation.^{67–69} These so-called “delay cells” are able to maintain firing across the delay period when information must be held “in mind” without sensory stimulation. In visuospatial working memory tasks, delay cells are spatially tuned, firing to the memory of one location but not others, and are able to hold the representation of information in working memory over a period of several seconds to perform the behavioral task.^{51,67} During adolescence, the proportion of dlPFC pyramidal cells that demonstrate delay-period activity significantly increases, suggesting functional recruitment of this cell-type in mediating working memory, which improves during the same period.⁵²

It should be noted that the primate dlPFC also contains other types of task-related neurons, including cells that fire to the Cue, or in anticipation of the motor response⁶⁷ (Figure 3). Most intriguingly, there are cells that fire during or just after the motor response (postsaccadic response “feedback” cells) that may be providing feedback about the response, i.e. corollary discharge from the motor system via mediodorsal thalamus.⁷⁰ These cells likely reside in layer V and are greatly influenced by DA D2 receptor stimulation.⁷¹ Dysfunction of these neurons may be very relevant to symptoms such as delusions and hallucinations, where loss of a proper mental tag that a thought is self-generated may lead to voices or thoughts being attributed to an external agent. However, as much less is known about these postsaccadic response feedback neurons, this review will focus on delay cells and the neurobiology of mental representations.

The groundbreaking work of Goldman-Rakic and colleagues defined the microcircuit basis underlying delay cell firing.⁵¹ Pyramidal cells in dlPFC deep layer III with similar spatial tuning properties engage in recurrent excitation to maintain the persistent firing across the delay period.⁵¹ Anatomical tract tracing experiments in nonhuman primates revealed that pyramidal cells in deep layer III have wide-spreading, horizontal axon terminals that terminate in a stripe-like pattern in the supragranular layers.^{72–76} This highly recurrent excitatory connectivity allows neuronal firing to continue within deep layer III microcircuits in the absence of sensory stimulation.⁷⁷ The spatial tuning properties are refined by parvalbumin-containing γ -aminobutyric acid (GABA) interneurons through lateral inhibition, thereby sculpting the pattern of activity within dlPFC layer III microcircuits^{78–83} (Figure 3).

These dlPFC layer III microcircuits expand during brain evolution, exhibiting striking differences in morphological, structural, and electrophysiological properties with the greatest expansion in human dlPFC.^{84–87} For example, there is an enormous expansion of the dendritic trees of layer III dlPFC pyramidal cells across evolution, and pyramidal cells of rhesus monkey layer III dlPFC have more than twice the number of spines as comparable neurons in primary visual cortex.^{84–87} In contrast, rodents have mPFC and orbital PFC but no dlPFC, with a large layer V and very small layer III.⁸⁸ Indeed, rodent PFC neurons are less complex than those in primates.⁸⁸ For example, comparative studies in mice have revealed that layer III pyramidal cells in frontal cortex and primary visual cortex (V1) have similar features in dendritic complexity, spine and excitatory synapse structure, and in electrophysiological metrics.⁸⁹ These findings lend credence to the notion that layer III microcircuits in rodents are cytoarchitecturally conserved with comparable physiological profiles. In contrast, layer III pyramidal cells in primates are fundamentally distinctive across brain regions, with the greatest dendritic complexity and the richest spine density in deep layer III of dlPFC.⁸⁴ The numerous spines on deep layer III dlPFC pyramidal cells are thought to subserve the extensive recurrent excitation that generate and sustain mental representations. In contrast, layer III pyramidal cells in primary sensory areas such as V1 are morphologically compact, have simple dendritic arbors, lower density of dendritic spines, and are characterized by higher input resistance, depolarized resting membrane potential, and higher action potential firing rates.⁹⁰ In addition, spontaneous excitatory postsynaptic currents (sEPSCs) are diminished in amplitude and frequency and have kinetic profiles in V1 faster than those in dlPFC layer III pyramidal cells.^{87,90,91} The neuronal networks in nonhuman primate V1 have a constrained dynamic range, thereby enabling faithful synaptic transmission. Recent comparative ultrastructural analyses have revealed that nonhuman primate dlPFC axospinous synapses are characterized by larger presynaptic boutons and postsynaptic densities compared to those in V1,⁹² consistent with a greater preponderance of dendritic spines in dlPFC compared to V1. Consequently, the electrotonically complex dlPFC layer III pyramidal cells in nonhuman primates have a much larger dynamic range, permitting integration of synaptic inputs of various amplitudes over changing temporal scales, allowing mental representations of multimodal information streams needed for higher-order cognition.^{88,93}

SPINE LOSS IN SCHIZOPHRENIA SELECTIVELY TARGETS DLPFC LAYER III MICROCIRCUITS

The microcircuits in dlPFC layer III that are necessary for mental representations are particularly susceptible in schizophrenia, and atrophy of these recurrent circuits likely contributes to a variety of symptoms, including cognitive deficits such as impaired working memory, thought disorder, and alogia. As described above, the onset of schizophrenia is accompanied by waves of dlPFC gray matter loss with cortical changes more pronounced in patients with shorter durations of prodromal symptoms^{26,94} and those associated with cannabis use.^{95,96} Studies conducted in postmortem tissue in subjects with schizophrenia have been particularly instrumental in elucidating the cellular, molecular, and circuit alterations in dlPFC layer III microcircuits (Figure 4). Neuropathological studies have identified significant atrophy in dlPFC deep layer III, including reduced neuropil.⁹⁷

Morphological studies using Golgi impregnation methods have revealed decreased dendritic arborization and profound dendritic spine loss (~25%) in dlPFC layer III pyramidal cells in schizophrenia,⁹⁸ while dendritic spine density in deeper cortical layers such as layer V appear to be unaltered within the same subjects.⁹⁹ Furthermore, somal volume of dlPFC layer III pyramidal cells are smaller compared to matched healthy subjects,¹⁰⁰ consistent with dendritic atrophy. There is likely a loss of the presynaptic compartment as well, as reflected by a decrement in various proteins localized in axon terminals.^{101–104} Importantly, these morphological alterations are far more pronounced in dlPFC than V1, as alterations in dendritic spine density and dendritic complexity were not significantly reduced in the same schizophrenia subjects in the layer III pyramidal cells in V1.⁹⁸ The loss of spine density in dlPFC was not attributable to antipsychotic medications or other potential confounds.

Postmortem studies have also revealed that there are various pre- and postsynaptic alterations related to GABA neurotransmission in schizophrenia, which would weaken lateral inhibition in dlPFC layer III microcircuits. A summary of alterations in GABAergic interneurons in the dlPFC of patients with schizophrenia is shown in Figure 4 (for detailed review, please see refs ^{105–107}). Although there was some consideration that insults to GABA interneurons may be a primary driver of disease, the more extensive evidence now suggests that impairments in dlPFC layer III pyramidal cells are a primary etiological disturbance in the disease and that GABA alterations are compensatory, by engaging in plasticity mechanisms to try to restore network activity.^{5,105} For example, the evidence now indicates that layer III dlPFC pyramidal cells are underactive in schizophrenia, not overactive, as would be predicted if GABA insults were the upstream cause.¹⁰⁸

A plausible consequence of hypoactive dlPFC layer III microcircuits also might explain changes in the DA system in schizophrenia, ultimately leading to reduced DA actions in cortex but increased DA actions in caudate, producing the canonical positive symptoms associated with the illness (Figure 5). Human imaging data suggest heightened DA D1R actions in dlPFC at the onset of disease¹⁰⁹ with a more complex picture at later stages with subcortical hyperdopaminergia and cortical hypodopaminergia.¹¹⁰ This supports previous studies suggesting that cognitive deficits in schizophrenia might be associated with reduced dopaminergic drive to the PFC.^{111,112} In fact, prior studies have shown that dlPFC activation is inversely related to the magnitude of striatal dopamine levels in schizophrenia patients.¹¹³

Research in animals suggests that PFC can regulate DA neuronal activity. High-resolution tract-tracing studies in rat brain have shown that PFC pyramidal cells innervate DA cells in the VTA in the ventral mesencephalon, which project back to the PFC.^{114–117} In contrast, PFC projections innervate the GABA interneurons in the VTA that project to the nucleus accumbens.¹¹⁵ These circuit diagrams in rat suggest that reduced PFC activity would weaken DA projections back to PFC, but disinhibit DA projections to striatum. Parallel connections may occur in primates, although the connections from any one subregion of PFC to the midbrain are more subtle.¹¹⁸ Reduced PFC activity may also augment DA release in caudate via disinhibition of striasomal output to DA neurons.^{119,120} Thus, there are a number of mechanisms whereby reduced firing of dlPFC pyramidal cells may produce both diminished DA activity in the PFC, and especially elevated activity in the caudate.¹¹⁰ Recent investigations in transgenic mouse models, which mimic some aspects of PFC insults

in schizophrenia, support this notion.^{121,122} Elevated DA stimulation of D2 receptors in caudate is well-established in schizophrenia¹²³ and may magnify cortical errors by weakening the inhibitory, corrective actions of the indirect pathway (Figure 5). Thus, a major action of D2R antagonist antipsychotic medications would be to diminish magnification of cortical errors. However, this mechanism would not fix the cortical alterations that may be the primary cause of disease.

In summary, the dlPFC is a critical locus of pathology in schizophrenia with wide-reaching consequence to brain physiology and mental state. Thus, illuminating the molecular mechanisms that regulate these newly evolved circuits may provide clues as to why these neurons are especially vulnerable to atrophy and may suggest plausible novel protective targets.

DYNAMIC NETWORK CONNECTIVITY OF DLPFC LAYER III: UNIQUE NEUROTRANSMISSION AND NEUROMODULATION

Recent studies have shown that dlPFC delay cells have unique physiological properties, including unusual mechanisms for neurotransmission and neuromodulation. In contrast to classic neuronal circuits, delay cell synapses rely mostly on acetylcholine to permit glutamate transmission, and their connections are weakened rather than strengthened by cyclic adenosine monophosphate (cAMP) signaling, e.g. during exposure to uncontrollable stress. Thus, they are particularly vulnerable to changes in arousal state. The unique regulation of layer III dlPFC glutamatergic synapses is summarized in Figure 6.

Neurotransmission at classic glutamate synapses, e.g. in V1, depends on AMPA receptor (AMPA) stimulation,¹²⁴ which depolarizes the membrane to relieve the Mg^{2+} block of *N*-methyl-D-aspartate receptors (NMDARs) to permit NMDAR actions. Classic synapses contain predominately NMDAR with GluN2A subunits, while those with slower, GluN2B subunits may reside outside the synapse. In contrast to these classic synapses, glutamate synapses in deep layer III of dlPFC are very different, with GluN2B subunit expressing NMDARs residing exclusively within the synapse and not at extrasynaptic sites.¹²⁵ At the physiological level, delay cell firing depends on glutamate stimulation of NMDAR with both GluN2A and GluN2B playing key roles, while AMPAR blockade has surprisingly little effect on cell firing.¹²⁵ GluN2B-containing NMDARs are particularly adapted to maintain DLPFC network firing in the absence of sensory stimulation due to the slower kinetics, where the faster kinetics of AMPARs can lead to dynamic instability and network collapse.¹²⁵ These findings are supported by computational modeling of neural dynamics.^{77,126–128} In contrast, postsaccadic response feedback cells in layer V require AMPAR stimulation for permissive opening of NMDARs, similar to canonical circuits in sensory and subcortical systems.¹²⁵ Consistent with this notion, transcript level expression of AMPARs is consistently higher in layer V pyramidal cells compared to that in layer III pyramidal cells in monkey dlPFC.¹²⁹

If AMPAR plays such a small role in delay cell firing, what provides the membrane depolarization needed for removal of the Mg^{2+} block of NMDARs? Both electrophysiological and immunoEM data show that these key permissive actions are

provided by cholinergic stimulation of nicotinic $\alpha 7$ receptors (nic- $\alpha 7$ Rs), which reside within the synapse.¹³⁰ These data are consistent with lesion data showing that cholinergic depletion from the dlPFC markedly impairs working memory performance in rhesus monkeys.¹³¹

In addition to these key cholinergic influences, dlPFC microcircuits are also powerfully modulated by the catecholamines, and depletion of catecholamines from the dlPFC is as harmful as lesioning the dlPFC itself.¹³² As schematized in Figure 7, both NE and DA have an inverted U influence on delay cell firing and cognitive performance, where moderate levels are essential to function, but excessive stimulation, e.g. during stress, suppresses neuronal firing and impairs cognitive abilities.³³ Moderate levels of NE enhance delay cell firing by activating postsynaptic $\alpha 2$ -A adrenoreceptors ($\alpha 2$ A-AR), while high levels of NE (such as those occurring during stress exposure) suppress delay cell firing through $\alpha 1$ adrenoreceptor ($\alpha 1$ -AR) stimulation.^{133,134} Similarly, either too little or too much DA D1R stimulation erodes delay cell firing.^{135,136} High levels of both NE and DA are released in the PFC during stress, rapidly taking PFC “off-line” to switch control of behavior to more primitive circuits that mediate habitual responses³³ (Figure 1B). Thus, the stress-induced impairments in PFC neuronal firing and working memory performance can be mimicked by stimulating $\alpha 1$ -AR or D1R in PFC and can be prevented by blocking these receptors.³⁴ Similar effects have been seen in humans, where stress impairs working memory and reduces dlPFC activity¹³⁷ and increases dopamine release in PFC.^{138,139} Human subjects with a weaker catechol-*O*-methyltransferase (COMT) Met-homozygote genotype were particularly vulnerable to stress-induced PFC dysfunction, consistent with a catecholamine dependent mechanism.¹⁴⁰

The molecular basis for these powerful catecholamine actions is now beginning to be understood, where these agents dynamically modulate feedforward cAMP–calcium signaling intracellular pathways in spines near NMDAR synapses to rapidly strengthen or weaken neural firing by altering the open state of nearby HCN and KCNQ potassium (K^+) channels⁴ (Figure 6). This phenomenon has been termed dynamic network connectivity.¹⁴¹ These signaling pathways provide a mechanism to rapidly alter connectivity based on arousal state⁴ and provide important negative feedback in a microcircuit with extensive recurrent excitation and lateral (rather than feedforward) inhibition. Thus, in contrast to classical circuits where cAMP–PKA signaling strengthens connections by increasing glutamate release and driving long-term plastic changes;^{124,142} in layer III of dlPFC, cAMP–PKA signaling rapidly weakens connectivity and reduces delay cell firing by increasing the open state of HCN and KCNQ channels.³⁴

ImmunoEM revealed a constellation of cAMP-related proteins in close proximity to the calcium-containing spine apparatus, the extension of the smooth endoplasmic reticulum (SER) into the spine, which are positioned to regulate feedforward cAMP–PKA–calcium signaling.¹⁴¹ Activation of cAMP–PKA can enhance internal calcium release through inositol-1,4,5-triphosphate receptors (IP3Rs)¹⁴³ and ryanodine receptors, while calcium release can activate protein kinase C (PKC) to further drive the production of cAMP,¹³³ thereby establishing a vicious, feedforward signaling process to activate K^+ channels and

weaken delay cell firing (Figure 6). These detrimental actions are induced by high levels of D1R and α 1-AR stimulation during stress exposure.^{133,144}

Conversely, a variety of mechanisms inhibit calcium–cAMP–K⁺ channel signaling in spines and strengthen connectivity to enhance cell firing (Figure 6). For example, both noradrenergic α 2A-AR and glutamatergic mGluR3 are concentrated on spines, where they enhance delay cell firing by inhibiting cAMP–K⁺ channel signaling.^{134,145} In contrast, mGluR2 are localized presynaptically, where they inhibit glutamate release.¹⁴⁶ Another critical regulator of feedforward cAMP–calcium signaling in newly evolved dlPFC layer III microcircuits is the phosphodiesterase, PDE4A, which catabolizes cAMP and is anchored to the spine apparatus by the scaffolding protein Disrupted in Schizophrenia (DISC1).^{147,148} Importantly, inflammation can reduce PDE4A actions and detach it from DISC1 via MAP kinase-activated protein kinase 2 (MK2) signaling.¹⁴⁹ Normally, PKA signaling provides negative feedback to reign in cAMP signaling by activating PDE4s.¹⁴⁹ However, this essential regulation is lost following MK2 activation,¹⁴⁹ indicating a mechanism whereby inflammation can dysregulate cAMP–calcium signaling and weaken layer III dlPFC synapses. These mechanisms also provide a nexus whereby psychological stress may act on a background of inflammation to weaken neural connections and induce atrophy of layer III dlPFC spines.

Repeated stress exposure produces dendritic atrophy and loss of dendritic spines in layer II/III pyramidal cells.^{150,151} These morphological changes are correlated with deficits in working memory in the delayed alternation task¹⁵² and cognitive flexibility on perceptual set-shifting tasks¹⁵³ and can be prevented blocking PKA¹⁵⁴ or PKC¹⁵² signaling, consistent with an underlying role of dysregulated DNC signaling. Dendritic atrophy and loss of spines are brain region-specific; in contrast to the PFC, chronic stress actually increases dendrite morphogenesis in the amygdala.¹⁵⁵ These findings lend credence to the notion that environmental stressors can detrimentally affect circuit and morphological properties of pyramidal cells, with the PFC being particularly vulnerable. Reduced PFC gray matter has also been seen in humans with repeated stress exposure.¹⁵⁶ It is possible that these mechanisms contribute to the loss of PFC gray matter as patients descend from the prodrome into psychotic illness during late adolescence and early adulthood.²⁶

RELEVANCE OF DYNAMIC NETWORK CONNECTIVITY TO SCHIZOPHRENIA

Perturbations to DNC mechanisms in dlPFC layer III microcircuits might play an important role in exacerbating susceptibility of these microcircuits in schizophrenia (Figure 8). Genome-wide association studies (GWAS) have identified a number of genes of relevance to layer III dlPFC synapses, including those associated with glutamate receptor transmission, HCN1 and calcium channels, and most recently, complement component 4A, which helps mediate synapse removal.^{18,157} Cognitive impairment in schizophrenia is particularly associated with genetic changes to signaling events associated with inflammation and oxidative stress.¹⁵⁸ Although the consequences of genetic alterations to protein expression/function and neuronal physiology are still requiring elucidation for most genetic associations, we can begin to create speculative bridges between what we are learning about

schizophrenia genetics, differences in transcript/protein expression postmortem, and the molecular regulation of layer III dlPFC synapses.

Copy number variation (CNV) studies, GWAS, and exome sequencing studies have particularly identified enrichment in the NMDAR-signaling and PSD protein complexes, which are central elements to regulating synaptic strength at glutamatergic synapses and thought to be associated with increased risk for developing schizophrenia.^{17,159–161} These genetic studies are also corroborated by postmortem evaluations showing reductions in various NMDAR subunits.^{162–164} There is also genetic evidence implicating the cholinergic system and nic- α 7Rs in the pathogenesis of schizophrenia.^{165–167}

Various lines of genetic and postmortem data implicate dysfunctional feedforward cAMP–calcium signaling intracellular pathways in the pathogenesis of schizophrenia. In general, schizophrenia is characterized by weakening of mechanisms that control cAMP–calcium signaling and strengthening of mechanisms that generate cAMP–calcium signaling (Figure 8). For example, GWAS established links between schizophrenia and the voltage-gated calcium channel Cav1.2 coded by *CACNA1C*, where genetic insults lead to increased calcium influx.¹⁶⁸ In cardiac muscle, calcium influx through this channel is increased by PKA phosphorylation¹⁶⁹ and results in increased calcium levels in the SER.¹⁷⁰ Similar actions in dlPFC neurons might exacerbate the stress response. Another gene linked to regulation of cAMP signaling is *DISC1*, which anchors PDE4s. Translocations in the *DISC1* gene have been associated with mental illness in a large Scottish pedigree and are thought to be an important risk factor predisposing individuals to schizophrenia;^{171,172} they have been implicated in several other neuropsychiatric illnesses that involve PFC dysfunction. In general, *DISC1* and its various protein interaction partners are thought to be a key hub for developmental pathways during neurogenesis and circuit maturation.^{173–175} Among the different proteins that *DISC1* tethers, a crucial interaction exists between *DISC1* and the phosphodiesterase PDE family of proteins.¹⁷¹ Our immunoEM analyses of layer III dlPFC spines have shown that *DISC1* anchors PDE4A next to the spine apparatus, positioned to regulate cAMP drive of calcium release.¹⁴⁸ Interestingly, genetic studies have also identified single-nucleotide polymorphisms (SNPs) in PDE proteins to be associated with schizophrenia that increase susceptibility.^{171,176} As described above, inflammation reduces PDE4 activity by preventing negative feedback by PKA and weakens PDE4 interactions with *DISC1*,¹⁴⁹ which may cause PDE4 mislocalization. Therefore, genetic or inflammatory insults could weaken *DISC1*-PDE4A modulation of feedforward cAMP–calcium signaling and lead to reduced neuronal firing and susceptibility to atrophy. Studies in rodents indicate that mutations in *DISC1* may only impact cognition under conditions of stress when PDE4 is needed to rein in elevated cAMP signaling.¹⁷⁷ Thus, environmental factors as well as mosaic expression of *DISC1* mutations in varying PFC subcircuits (e.g., dlPFC circuits, schizophrenia; medial PFC circuits, depression) may help to explain the heterogeneity in symptomology with *DISC1* genetic alterations.

Another intracellular mediator of feedforward cAMP–calcium signaling is Regulator of G protein signaling 4 (RGS4) that normally inhibits G protein (especially G_q) signaling and regulates NMDAR function. RGS4 is concentrated next to excitatory synapses on spines in layer III primate dlPFC.¹⁷⁸ RGS4 mRNA and protein levels are downregulated in the dlPFC

in subjects with schizophrenia,^{179–181} and these changes are most prominent in dIPFC layer III pyramidal cells.¹⁸² Genetic association and linkage studies have also identified several SNPs for RGS4 at the genomic level.¹⁸³ If RGS4 inhibits Gq signaling near the synapse in layer III dIPFC, the downregulation of RGS4 in schizophrenia may disinhibit calcium signaling and weaken layer III dIPFC synapses.

Finally, the group II metabotropic glutamate receptor, mGluR3, which couples to G_i/G_o and inhibits cAMP–calcium signaling, has also been genetically linked to schizophrenia^{184,185} including GWAS confirmation.¹⁸ Postmortem studies have also shown evidence of reduced mGluR3 actions in the dIPFC of patients with schizophrenia, including increased expression of glutamate carboxypeptidase II (GCP II), the catabolic enzyme that destroys the endogenous mGluR3 ligand, *N*-acetylaspartylglutamate (NAAG).¹⁸⁶ Recent studies in monkeys show that mGluR3 plays an expanded role in the primate dIPFC. In addition to its classic location in astrocytes, mGluR3 is localized postsynaptically on spines in monkey dIPFC layer III microcircuits, where its stimulation enhances dIPFC delay cell firing through inhibition of cAMP signaling.¹⁴⁶ Conversely, maternal inflammation has been shown to increase microglial GCP II expression in neonate brain, which would reduce endogenous mGluR3 stimulation by NAAG.¹⁸⁷ Taken together, these studies suggest that various DNC mechanisms that normally serve to inhibit feedforward cAMP–calcium signaling in dIPFC layer III microcircuits may be reduced in schizophrenia subjects.

Some DNC mechanisms that normally generate feedforward cAMP–calcium signaling are increased in schizophrenia (Figure 8). For example, schizophrenia subjects are associated with elevated expression levels of group I metabotropic glutamate receptors (i.e., mGluR1 α), which elicits greater intracellular Ca²⁺ release.¹⁸¹ ImmunoEM experiments in monkey dIPFC layer III microcircuits have also revealed that mGluR1 α are localized both presynaptically and on dendritic spines and are positioned to mobilize Ca²⁺ from intracellular stores.¹⁸⁸ Similarly, in vivo imaging studies have revealed that drug-naïve and drug-free subjects show elevated levels of dopamine D1 receptor (D1R), which has been hypothesized to compensate for lower available dopamine in the dIPFC in subjects with schizophrenia^{109,189} but may lead to excessive D1R signaling during adolescence when DA levels may be especially high in layer III.^{36,37}

Exacerbated feedforward cAMP–calcium signaling might also be associated with dysregulation of actin cytoskeleton pathways and mitochondrial function, e.g. due to toxic calcium dysregulation (Figure 4). In addition to its anchoring of PDE4 enzymes, DISC1 anchors Kalirin-7, a RAC guanine nucleotide exchange factor (GEF) that is highly concentrated in dendritic spines and regulates spine integrity through RAC1 and cell division cycle (CDC42).^{175,190} Previous postmortem studies in schizophrenia have revealed lower mRNA levels of Kalirin-7 and CDC42, and these changes were also correlated with spine density measures in dIPFC deep layer III pyramidal cells.¹⁹¹ The *KALRN* locus is associated with risk for schizophrenia¹⁹² and missense mutations have also been reported in a small cohort.¹⁹³ Various actin cytoskeleton signaling pathways pertinent to CDC42 (e.g., CDC42–CDC42EP, CDC42–PAK–LIMK, and CDC42–NWASP–ARP2/3) are perturbed selectively in dIPFC layer III pyramidal cells in schizophrenia.^{121,191,194,195} In addition, de novo mutations in schizophrenia are overrepresented among loci encoding cytoskeleton-

associated proteins that modulate actin filament dynamics, actin bundle assembly, interconnected with activity-regulated cytoskeleton-associated protein (ARC) and NMDAR signaling and could contribute to the pathogenesis of the illness.¹⁵ Impairments in actin cytoskeleton signaling pathways that are necessary to maintain mature dendritic spines, is predicted to destabilize dendritic spines, leading to spine loss.⁵ It is plausible that intrinsic genetic predisposition is modulated by mosaic expression and/or region and cell-type specific patterns of gene expression changes, to influence the selective vulnerability of dlPFC layer III microcircuits in schizophrenia.

In accordance with these findings, recent cell-type specific studies in dlPFC layer III pyramidal cells in schizophrenia have revealed downregulation of nuclear gene products regulating mitochondrial and ubiquitin–proteosomal signaling pathways.¹⁰⁸ The molecular alterations germane to mitochondrial energy production and regulation of protein translation appear to be distinctive for schizophrenia as these transcriptome signatures are not observed in bipolar disorder or major depression.¹⁹⁶ These neuropathological changes might be driven, at least in part, by aggravated feedforward cAMP–calcium signaling by inducing calcium leak from the smooth endoplasmic reticulum in a PKA dependent manner. This corroborates findings suggesting that dlPFC microcircuits in schizophrenia are hypoactive,^{45,197,198} with a greater propensity to affect layer III pyramidal cells. The vicious interaction between exaggerated feedforward cAMP–calcium signaling, actin cytoskeleton impairments and mitochondrial dysfunction might also shed light on attenuated γ -frequency neural oscillations (30–80 Hz) in patients with schizophrenia.^{199–202} Recordings from monkeys show that γ -frequency neural oscillations are associated with the persistent firing of dlPFC delay cells during working memory,²⁰³ possibly linking reduced γ -oscillations to weaker dlPFC layer III microcircuits. Consistent with this notion, the deficits in γ -frequency neural oscillations in schizophrenia are more pronounced in the dlPFC compared to those in posterior regions.²⁰⁴

Finally, we should add that genetic and/or environmental insults to the presumed layer V response feedback cells in primate dlPFC may also contribute to symptoms of schizophrenia. These cells appear to relay the “corollary discharge” that a response is being carried out by brain circuits. These cells are greatly influenced by AMPAR¹²⁵ and dopamine D2 receptor (D2R) signaling.⁷¹ Alterations in these molecular events in response feedback cells may thus disrupt the mental tag that a neural event was self-generated, contributing to delusions and hallucinations. This is an important arena for future research.

HYPOTHETICAL CASCADE OF EVENTS IN THE PATHOGENESIS OF SCHIZOPHRENIA

The “dual-hit” working model of schizophrenia posits that insults to brain development in utero are compounded by a second wave of assaults during late adolescence to cause a descent into illness (Figure 2). A variety of genetic and environmental insults in utero and during early perinatal development could impair cortical circuit formation and maturation and set up an inflammatory framework that could exacerbate later responses to environmental insults (e.g., stress), causing atrophy of dlPFC circuits, profound dlPFC

dysfunction, and the full emergence of schizophrenia symptoms. PFC circuits are particularly susceptible to environmental perturbations (e.g., stress) given the highly complex and protracted maturation, providing greater opportunities for insults to amplify alterations in developmental trajectories. For example, dysregulated feedforward cAMP–calcium signaling during stress could drive abnormal mitochondrial signaling, leading to actin cytoskeleton collapse and further activation of inflammatory pathways, establishing vicious cycles that result in atrophy of deep layer III spines. Increased vulnerability during adolescence might be compounded by genetic insults that impact synaptic pruning mechanisms and circuit reorganization. For example, variations in the major histocompatibility (MHC) locus and complex structural variations in complement C4 are strong contributors to schizophrenia risk.¹⁵⁷ The elevated expression levels of C4 in schizophrenia subjects, in turn, might promote excessive pruning of spines and synapses during adolescence.¹⁵⁷ Thus, gene–environment interactions appear to be a key factor in precipitating illness onset for patients with schizophrenia. Elucidating the neural underpinnings of dlPFC circuit alterations in nonhuman primates and in postmortem tissue in schizophrenia patients, might provide insight into pathophysiological impairments such as lower power of γ -frequency oscillations and working memory deficits.

RELEVANCE OF DNC MECHANISMS IN DLPFC LAYER III TO NOVEL THERAPEUTIC TREATMENTS FOR SCHIZOPHRENIA

Interrogation of DNC mechanisms in nonhuman primates and rodents, suggest that there are several plausible targets that might be pursued for the development of appropriate therapeutic targets to protect layer III dlPFC circuits during the prodrome. One key neuroprotective mechanism involves inhibition of cAMP–PKA signaling with α 2A-AR agonist guanfacine. Although the neuroprotective mechanisms of guanfacine arise from several plausible routes, the primary mechanism revolves around stimulation of α 2A-ARs on layer III spines, through inhibition of cAMP–HCN channel signaling.¹³⁴ Electrophysiology studies in nonhuman primates show that α 2A-AR inhibition of cAMP–HCN signaling enhances dlPFC firing,¹³⁴ and either intra-PFC or systemic treatment with guanfacine strengthens PFC function.²⁰⁵ Particularly pertinent to schizophrenia, daily guanfacine treatment is also protective of PFC dendritic spines and cognitive functions in a chronic restrained stress paradigm,¹⁵⁴ or from hypoxia,²⁰⁶ in rat medial PFC, encouraging this approach. As α 2A-AR-agonists such as guanfacine are also anti-inflammatory, deactivating microglia,²⁰⁷ they may also be more generally protective of brain during the prodrome. As guanfacine is FDA approved for use in children 6–17 years, it would be feasible to test in teens at risk for schizophrenia.

Enhancing cholinergic actions in layer III dlPFC microcircuits might also strengthen network activity. For example, there has been a longstanding interest in the role of nic- α 7Rs as a potential treatment strategy for schizophrenia.¹⁶⁶ The nic- α 7Rs are enriched in NMDAR glutamate network synapses and are permissive for NMDAR communication in dlPFC layer III pyramidal cell circuits.¹³⁰ Administration of low-doses of nic- α 7R agonists enhances delay cell firing and improves working memory, but higher doses produced excessive neuronal excitability and no longer improved working memory.¹³⁰ Thus, it is

essential to test very low doses in human studies. These findings might also shed light on why schizophrenia patients have a greater propensity for tobacco dependence, with over 85% of hospitalized schizophrenia patients engaging in smoking due to potential beneficial effects on cognitive function.^{208–210} Recent quantitative trait loci and gene expression analyses with high-resolution 3D chromatin contact maps during corticogenesis implicate acetylcholine as a plausible etiological mechanism in the pathogenesis of schizophrenia.¹⁶⁷ In addition, previous studies suggest that muscarinic M1Rs might also be positioned in dlPFC layer III glutamate synapses to enhance spatial representation.²¹¹ Ongoing studies in nonhuman primates are delineating the relative contribution of M1Rs to persistent firing and if these receptors can be targeted by novel agonists and positive allosteric modulators (PAMs).

Finally, another possible candidate involves enhanced modulation of the metabotropic glutamate receptor mGluR3. As described earlier, mGluR3 are concentrated on layer III spines, while mGluR2 are mostly presynaptic in primate layer III dlPFC.¹⁴⁶ Postsynaptically localized mGluR3 are activated by both glutamate and NAAG, which are both released from glutamate terminals.²¹² Physiological recordings from monkeys show that NAAG stimulation of mGluR3 in dlPFC greatly enhances delay cell firing by inhibiting cAMP–K⁺ channels signaling.¹⁴⁵ NAAG activity is inhibited by the NAAG peptidase GCPII.²¹³ A NAAG peptidase inhibitor markedly increased the firing of delay cells,¹⁴⁶ suggesting these agents may have therapeutic potential. As postmortem studies have revealed increased protein levels of GCPII and reduced levels of mGluR3 in the dlPFC in subjects with schizophrenia,¹⁸⁶ GCPII inhibitors might be an effective approach to increase endogenous NAAG levels to curb feedforward cAMP–calcium signaling. Low doses of mGluR2/3 agonists that preferentially engage postsynaptic actions also enhance delay cell firing and improve working memory in monkeys,¹⁴⁵ and a meta-analysis of mGluR2/3 agonist effects in patients with schizophrenia has shown that low (but not high) doses of mGluR2/3 agonists can be beneficial in patients in early stages of the illness.²¹⁴ Thus, data from patients with the illness encourage this strategy.

There are many fundamental challenges in drug discovery, including (1) differential drug effects in various brain regions, (2) refinement of drug dosage given inverted U-shaped drug response curves, and (3) a changing landscape of the disease process, where therapeutic targets may be lost (e.g., from spines) in later stages of disease. These putative pharmacological targets hold promise for protecting dlPFC circuits and preventing the emergence of symptoms of the illness. Because the dlPFC is a central locus of pathology in a range of neurological disorders, the putative therapeutic targets outlined in this review might also be suitable for treating age-related cognitive decline and neurodegenerative diseases (e.g., Alzheimer's disease). Strategies for synergistic application of pharmacological intervention along with cognitive remediation and psychiatric rehabilitation²¹⁵ might further enhance neuropsychological performance for subjects with schizophrenia.

CONCLUSIONS

The same molecular machinery that allows extraordinary flexibility in layer III dlPFC network connectivity may also confer vulnerability to atrophy when dysregulated by genetic perturbations and/or environmental insults. In contrast to the classical sensory circuits, the molecular mechanisms that govern network connectivity in the dlPFC may be particularly susceptible to stress exposure, as signaling events weaken connectivity. Signaling interactions in dlPFC layer III dendritic spines may provide a nexus whereby inflammation disinhibits the neuronal response to physiological and/or psychological stress, which may weaken connections at multiple stages of brain development. Therefore, understanding the unique regulation of these circuits may help to identify novel therapeutic strategies to protect circuits during the early stages of illness.

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Figure 1.

Differential sensitivity of neuronal circuits during stress. The newly evolved PFC subserves many higher cognitive functions, including abstract reasoning, executive functions such as planning and organization, and metacognitive operations, including insight about errors in thought and action. The PFC has extensive connections that allow top-down regulation of brain function. The PFC is also organized in a topographical manner with dorsal and lateral regions mediating thought and action, while ventral and medial regions mediate emotion. The PFC also has extensive interconnections with the arousal systems, including NE and DA cells in the brainstem. Panel A: Under alert, nonstressed conditions, there are optimal levels of catecholamine release in PFC, allowing top-down regulation of cortical and subcortical structures and strong cognitive function. Panel B: Under conditions of uncontrollable stress, high levels of catecholamine release take the dlPFC “offline”, while simultaneously strengthening subcortical and sensory cortical functions. Chronic stress exposure induces architectural changes, i.e. atrophy of layer III PFC connections. These actions may be relevant to dlPFC gray matter loss in schizophrenia.

Figure 2.

Cumulative impact of genetic predispositions and stress in the pathogenesis of schizophrenia. Schizophrenia is thought to arise from a dual hit, whereby perinatal, developmental insults set the stage for later neuropathological changes in adolescence. Healthy neuronal circuits (black line) undergo a stereotypical developmental pattern, whereby the number of excitatory connections via dendritic spines increase into childhood and then undergoes a normative pruning process. Increased risk of schizophrenia (blurred blue line) is associated with a myriad of environmental perturbations during gestation, including (1) environmental factors such as immune activation, infection, and malnutrition that may drive neuroinflammation and (2) genetic insults that impair synapse and circuit maturation. A second hit is seen in adolescence with an acceleration of gray matter loss at descent into illness. DNC regulation of deep layer III dlPFC connections may render these synapses particularly vulnerable to atrophy from physiological and psychological stress, especially given the increased DA innervation of layer III in adolescence. In healthy individuals, these trajectories are tightly regulated by molecular mechanisms (e.g., PDE4s), but in schizophrenia patients, a background of inflammation (genetic and/or environmental) would dysregulate stress signaling and drive circuit modifications, ultimately resulting in gray matter loss. Symptoms would emerge when synaptic connectivity decreases below a hypothetical threshold (illustrated by red dashed line) needed to support normal functioning.

Figure 3.

The dorsolateral prefrontal cortex and neuronal circuits mediating spatial working memory. Panel A: Schematic summarizing the oculomotor delayed response (ODR) task used to probe spatial working memory and the involvement of different cell-types in the dIPFC using electrophysiology measures. For each trial of the ODR task, the rhesus monkey must fixate on a central point and remember the spatial position of a cue in 1 out of 8 locations. Following a delay period of multiple seconds, the monkey must indicate the position with a saccade to the memorized spatial location. The cued position randomly changes over a plethora of trials. Panel B: Schematic illustration of the microcircuits within monkey dIPFC (Walker's area 46) where neurons show persistent, spatially tuned firing during the delay period of the ODR task. Examples of two types of task-related neurons are shown: a delay cell with persistent firing for a preferred spatial location, and a postsaccadic response feedback cell. The dIPFC receives a large variety of inputs, including highly processed visuospatial information from the parietal association cortex and a variety of neuromodulators such as dopamine (green). Delay cells are thought to reside within microcircuits in deep layer III with extensive recurrent excitatory connections among neurons with shared preferred directions. This recurrent excitation is mediated by NMDAR (GluN2A and GluN2B) synapses on dendritic spines with marginal contribution from AMPARs. The spatial tuning activity of dIPFC layer III delay cells is sculpted by lateral inhibition from GABA interneurons, particularly involving parvalbumin-containing basket and chandelier cells. It is hypothesized that delay cells send their information to the motor systems via presaccadic response cells, and that postsaccadic response cells receive feedback

(corollary discharge) regarding the motor response. Postsaccadic response cells depend on AMPAR as well as NMDAR stimulation and are powerfully modulated by DA D2R stimulation; as D2R expressing neurons are focused in layer V, it is likely that these cell types reside in dlPFC layer V.

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Figure 4.

Alterations in dlPFC layer III microcircuits in schizophrenia. Schematic summarizing abnormalities observed from neuropathological postmortem studies in schizophrenia subjects.^{5,105–107} Inspired by the pioneering work from Lewis and colleagues and other groups, it has been surmised that the primary disturbance in dlPFC layer III microcircuits in schizophrenia lies in decreased recurrent excitation between pyramidal cells. Consistent with this notion, dlPFC layer III pyramidal cells (blue) in subjects with schizophrenia are characterized by numerous morphological changes, such as decreased density of dendritic spines, reduced dendritic arborization and decreased somal volume.^{98,100} Furthermore, dlPFC layer III pyramidal cells are characterized by molecular changes involving impairments in the actin cytoskeleton^{121,194} (e.g., CDC42, ARP2/3 signaling pathways) and mitochondrial function.¹⁰⁸ To restore network activity, the upstream alterations in pyramidal cells are accompanied by compensatory changes in GABA interneurons (red) to reduce lateral inhibition. For example, the decreased activity of pyramidal cells is thought to reduce excitation of parvalbumin basket (PVb) cells through various activity-dependent mechanisms involving GAD67, PV, Zif268, and PNNs.^{216–219} Furthermore, dysregulated splicing of ERBB4 and lower expression of dysbindin may contribute to compromised excitatory drive to PVb cells.^{220–222} Additional changes to GABA interneurons include deficits in PV chandelier cells (PVCh), SST, and CCK cells.^{223–225} A downstream consequence of changes in excitation–inhibition in dlPFC layer III are changes in dopamine transmission, whereby dlPFC microcircuits receive reduced mesocortical dopamine innervation.¹⁸⁹ These dopaminergic changes are associated with compensatory but insufficient upregulation of D1Rs.¹⁰⁹ CDC42, cell division cycle 42; ARP2/3, actin-related protein 2/3; GAD67, glutamic acid decarboxylase 67; PV, parvalbumin; PNN, perineuronal

nets; SST, somatostatin; NPY, neuropeptide Y; CCK, cholecystokinin; CB1, cannabinoid receptor 1; GAT1, GABA transporter 1; ERBB4, receptor tyrosine-protein kinase erbB-4; D1R, dopamine receptor D1.

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Figure 5.

Cascade of changes in schizophrenia across cortical and subcortical systems. Healthy subject: Pyramidal cells in the dlPFC regulate DA neurons projecting to striatum via several mechanisms (see text) and convey information directly to the caudate nucleus as part of cortical-basal ganglia circuits mediating cognition. The indirect pathway emerging from the striatum normally serves to inhibit inappropriate thoughts and actions and performs this function under conditions of normal levels of DA release. Schizophrenia: Insults to dlPFC circuits cause errors in cortical processing. Hypoactive pyramidal cells in the PFC may also disinhibit DA release in caudate, inducing excessive DA stimulation of D2R, which inhibits the indirect pathway. The loss of indirect pathway regulation would magnify cortical errors, intensifying cognitive impairment and likely contributing to positive symptoms such as delusions and hallucinations. Antipsychotic medications with D2R-blocking actions would restore indirect pathway regulation and reduce symptoms but not correct the underlying errors.

Figure 6.

DNC in primate dlPFC layer III. Schematic summarizing the molecular regulators of DNC in dlPFC layer III NMDAR synapses on long, thin spines that permit biochemical/electrical compartmentalization of signaling. These synapses are mediated by NMDAR with either GluN2A or GluN2B subunits. In primate dlPFC, the permissive depolarization for NMDAR-containing synapses is provided by powerful influences of acetylcholine through nicotinic $\alpha 7$ receptors (nic- $\alpha 7$ R). Negative feedback in these recurrent circuits is provided by feedforward calcium-cAMP-PKA signaling, which open HCN and KCNQ channels near the synapse to weaken connectivity. Internal calcium release from the spine apparatus (i.e., the SER in the spine) may be initiated by calcium influx through NMDAR by activation of type I metabotropic glutamate receptors (e.g., mGluR1 or mGluR5) activation of Gq signaling or by catecholamine activation of Gq or Gs-cAMP signaling, e.g. as occurs during stress exposure. RGS4 is positioned to regulate Gq signaling near the synapse. A variety of mechanisms regulate cAMP signaling. For example, moderate levels of norepinephrine release during nonstressed conditions engage high affinity $\alpha 2$ A-ARs that inhibit cAMP opening of HCN and KCNQ channels to strengthen connections and enhance cognition. Recent data show that postsynaptic mGluR3 also performs this key strengthening action. cAMP signaling is also powerfully regulated by PDE4A, which is anchored to the spine apparatus by DISC1. As the spine apparatus interconnects with the SER in the dendrite, these mechanisms also allow communication between the synapse and the dendrite, e.g. with mitochondria residing in the nearby dendrite. Thus, regulation of feedforward cAMP-calcium signaling mechanisms is crucial to maintain normal mitochondrial function and the actin dynamics needed for the structural integrity of dendritic spines. Abbreviations: NMDAR, *N*-methyl-D-aspartate receptor; mGluR, metabotropic glutamate receptor; ACh, acetylcholine receptor; $\alpha 2$ -AR/ $\alpha 1$ -AR, $\alpha 2$ -A/ $\alpha 1$ adrenoreceptors; D1R, dopamine receptor D1; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PKC, protein kinase C; AC, adenylyl cyclase; RyR, ryanodine receptor; IP3R, inositol-1,4,5-triphosphate

receptors; RGS4, regulator of G protein signaling 4; PDE4A, phosphodiesterase 4A; DISC1, Disrupted in Schizophrenia 1.

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Figure 7.

Impact of neuromodulators in dlPFC physiology and function. Schematic illustration of the inverted U-shaped influence of NE, ACh, and DA on the memory fields of dlPFC delay cells representing a position in visual space (blue = low firing, red = high firing). During deep sleep, no modulators are released, and dlPFC synapses are not able to communicate (unconscious). With drowsy waking, low levels of ACh and DA permit NMDAR in the postsynaptic density to respond to glutamate, but signaling is weak and noisy. Under optimal conditions, moderate levels of NE release engage $\alpha 2$ A-ARs to inhibit cAMP- K^+ channel signaling and strengthen persistent firing for the preferred direction. Moderate levels of DA D1R stimulation also reduce "noise", and along with lateral inhibition, produce more refined representations. However, high levels of catecholamine release with uncontrollable stress engage $\alpha 1$ -ARs and D1R to suppress all delay cell firing, eroding mental representations.

Figure 8.

Aberrant DNC mechanisms in schizophrenia dIPFC layer III. Schematic summarizing changes in DNC mechanisms in schizophrenia dIPFC layer III due to genetic predispositions and neuropathological changes from postmortem studies. For example, both genetic and postmortem studies have revealed a decrement in acetylcholine nic- α 7R and glutamate NMDAR signaling. Translocations in the *Disc1* gene have been implicated in mental illness and in particular schizophrenia. DISC1 serves as a scaffold for the family of phosphodiesterases (e.g., PDE4A) that hydrolyze cAMP signaling, which have also been genetically linked to schizophrenia. ImmunoEM studies of monkey dIPFC layer III spines show that DISC1 anchors PDE4A near the spine apparatus, adjacent to HCN channels. In vitro studies show that PDE4 activity is inhibited by MK2 signaling during inflammation. If similar actions occur in human PFC in response to inflammation, e.g. in utero and/or during adolescence at the onset of illness, dysregulated cAMP–calcium signaling may lead to weaker connections and loss of spines. Schizophrenia is also associated with elevation in mGluR1 α which drives internal calcium release. The activity of mGluR1 α is inhibited by regulator of G protein signaling 4 (RGS4), positioned perisynaptically to gate signaling. Recent layer III pyramidal cell-type specific studies have also revealed downregulation of RGS4 mRNA in schizophrenia, which is predicted to result in dysregulated feedforward cAMP–calcium signaling. Finally, neuroimaging studies have suggested elevation in D1R in drug naïve patients which would further exaggerate a vicious cycle. These perturbations are thought to converge of impairments in actin cytoskeleton and mitochondria in dIPFC layer III pyramidal cells in schizophrenia, leading to enhanced vulnerability and weakening of network connectivity. In sum, various studies in schizophrenia suggest alterations that

increase the generation of cAMP–calcium signaling and decrease mechanisms that moderate cAMP–calcium signaling.

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