Signal Transduction and Genes-to-Behaviors Pathways in Psychiatric Diseases

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Although severe psychiatric diseases such as schizophrenia are among the most common and destructive of all human illnesses and have a major genetic component, the molecular and cellular mechanisms underlying their complex pathophysiology remain to be fully elucidated. Two recent papers, one using behavioral analysis of knockout mice and one using allele associations in patients with schizophrenia, both suggest that the protein phosphatase calcineurin—a protein at the nexus of multiple signaling cascades—may play a role in the pathophysiology of schizophrenia. In this Perspective, we evaluate evidence suggesting that signaling pathways are likely central contributors to the pathophysiology of psychiatric illnesses. We follow with a discussion of the possible mechanisms by which aberrant calcineurin signaling may contribute to some facets of the complex clinical presentation of schizophrenia. We present a minimal orientation on schizophrenia to facilitate the crossing of disciplines (*1*) and the integration of knowledge by those readers who may be unfamiliar with the phenomenology by which specialists classify a person as suffering from schizophrenia.

A primer on schizophrenia: Rationale for studying signaling pathways in psychiatric disorders

Severe mental disorders diagnosed on the basis of gross impairments in contact with reality and manifested by delusions (false beliefs), hallucinations (false perceptions from the senses of vision, hearing, touch, or smell), and disordered thinking (inferred from speech content and patterns) are termed psychoses. One relatively distinctive psychosis is schizophrenia; others include bipolar disorder (formerly manic-depressive disease), certain forms of major depressive disorder, delusional disorder (formerly paranoia), and a group of disorders that are secondary to exposure to various drugs available on the street and prescribed drugs and to brain injuries and diseases (*2*). Paradoxically, schizophrenia is diagnosed primarily by its configural psychopathology on the basis of symptom profiles just as it was at the beginning of the 20th century; no neuroscience correlates yet provide useful enough criteria. Perhaps the recent findings implicating calcineurin (*3*, *4*) and other important genes such as neuregulin-1, dysbindin, catechol-*O*-methyltransferase, G72, and RGS4 [for a review, see (*5*)] in the pathogenesis of schizophrenia will provide the basis for a new diagnostic and classification system. Schizophrenia afflicts about 1% of the adult populations of the world—industrialized or developing, rich or poor, males and females equally—although the age of onset, generally ranging from puberty to the early fifties, is 4 to 5 years earlier in males, and the disease has a variable course with respect to severity and number of episodes. There is no doubt, based on data from genetic epidemiology (family, twin, and adoption strategies), that genetic factors are important and major contributors to the distal etiology (*6*). Further, there is no doubt that various prenatal, perinatal, and postnatal nongenetic contributors are involved in the complex clinical phenotypes of schizophrenia (*7*), as are epigenetic (*8*, *9*) and stochastic factors (*10*). The devil is in the details. Similar to other common diseases with important non-Mendelian genetic influences and multiple nongenetic contributors, schizophrenia is a complex disease (compare cancers, heart disease, diabetes I and II, and their ilk) (*11*). A consensus has emerged that a dynamic "diathesis × stressor" model that allows for the interaction between predisposition and environment within the context of systems biology, in which information from many levels must be integrated (Fig. 1), will provide the most guidance for ongoing research (*12*). Guarded optimism for advances on the gene end of the gene-to-behavior pathway arises from replications and strong findings for some candidate genes and gene regions (*5*, *13*, *14*) (Fig. 1). Further development of animal models for these gene to behavior pathways and their regulation are essential to progress in understanding the psychoses and other psychiatric disorders (*3*, *4*, *15-17*).

Can complex neuropsychiatric disorders actually arise from abnormalities in intracellular signaling pathways?

Evidence suggests that alterations in critical intracellular signaling pathways have an important role in the pathophysiology and treatment of complex neuropsychiatric disorders (*18*) (Fig. 2). This evidence comes from many levels, including the finding that the most effective medications for treating these disorders have immediate, acute, and long-term effects on signaling pathways. Signaling pathway components are direct targets of some psychotropic medications. For example, the most well established treatment for bipolar disorder, lithium, is believed to exert its initial biochemical effects by inhibiting the activity—through competition for magnesium—of a select group of enzymes, including inositol monophosphatase (IMPase) and glycogen synthase kinase-3 (GSK-3) [see (*19*, *20*) for reviews]. Another mood stabilizer, valproic acid, likewise inhibits the activity of some enzymes [for example, succinate semialdehyde dehydrogenase (*21-25*) and histone deacetylases (*26*, *27*)] and, in a way similar to lithium, may exert its therapeutic effects by direct modulation of signaling pathways [see (*25*, *28*, *29*) for reviews]. The vast majority of other psychotropic medications exert direct effects either on membrane receptors or on neurotransmitter reuptake, thereby leading to indirect effects on intracellular signaling. For instance, cyclic adenosine

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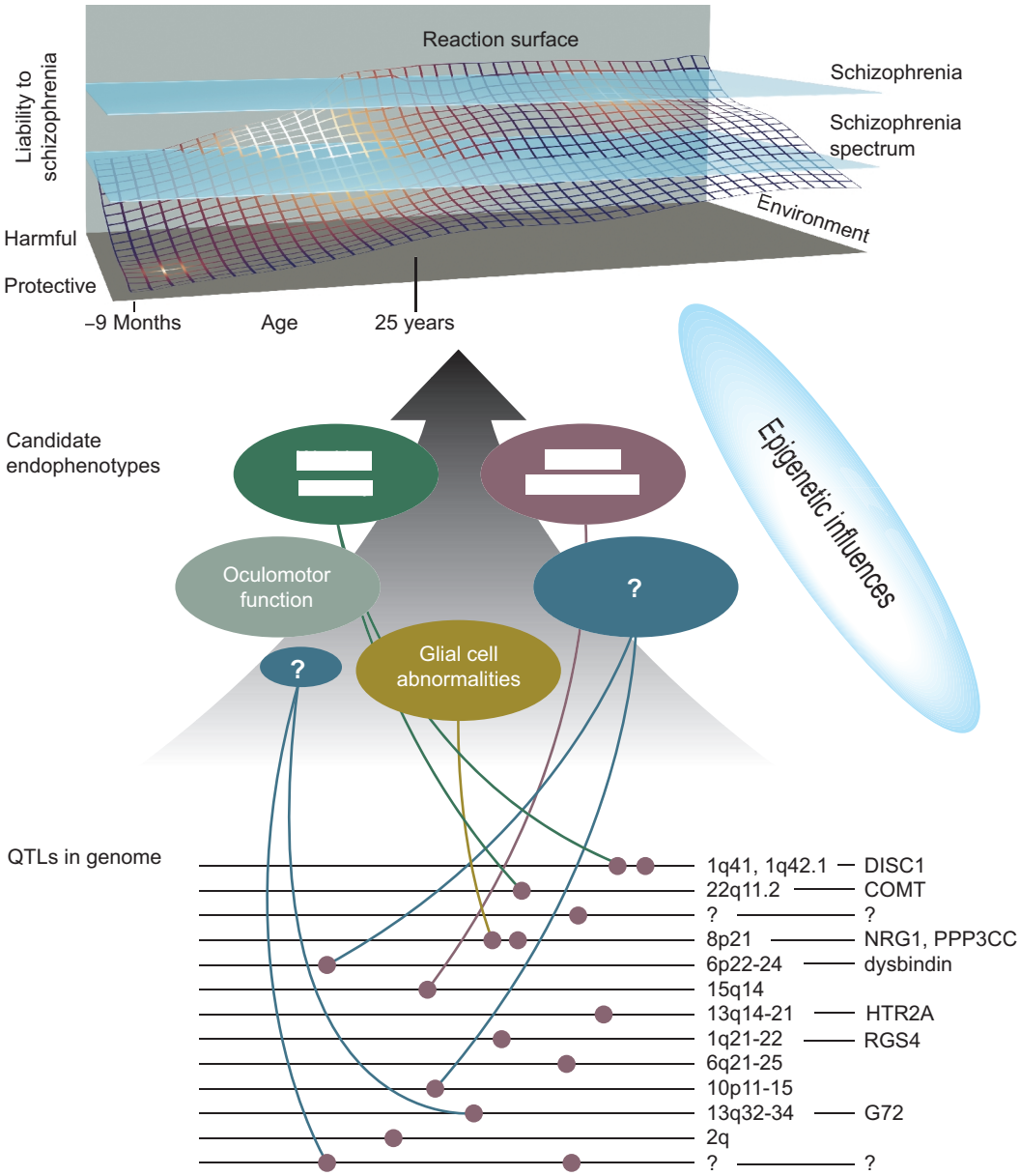

Fig. 1. A systems approach to understanding schizophrenia, showing the involvement of candidate gene regions, candidate genes, and putative candidate endophenotypes, and allowing for the influence of pre- and postnatal environmental and epigenetic factors over the course of development. Numbers at lower right are chromosome locations currently identified, and abbreviations are those for genes, if known, at those locations; see (*12*) for details. Question marks symbolize gaps in our knowledge. Blue planes intersecting the Reaction surface indicate levels of liability, above which a diagnosis is called for. QTL, Quantitative trait loci. [Adapted from (*12*), © 2003 I. I. Gottesman and used with permission]

monophosphate (cAMP) is targeted through the blockade of dopamine receptors by antipsychotics, and selective serotonin reuptake inhibitors (SSRIs) lead to modulation of serotonin heterotrimeric GTP-binding protein (G protein) coupled receptors (GPCRs). Even the efficacy of novel psychotropics (currently in initial clinical trials) such as corticotrophin-releasing hormone (CRH) antagonists, glucocorticoid receptor antagonists, and neuropeptide modulators rely on the modulation of signaling pathways (*30-33*). Further, because the vast majority of psychotropic medications (with the exception of acutely acting sedatives and anxiolytics) exert their primary therapeutic actions only following week(s) of treatment, these therapeutic effects are believed to involve downstream changes in gene and protein expression that are initiated and maintained by critical intracellular signaling pathways (*34-36*).

Psychiatric disorders are characterized by a diverse constellation of signs and symptoms. In addition to the diverse

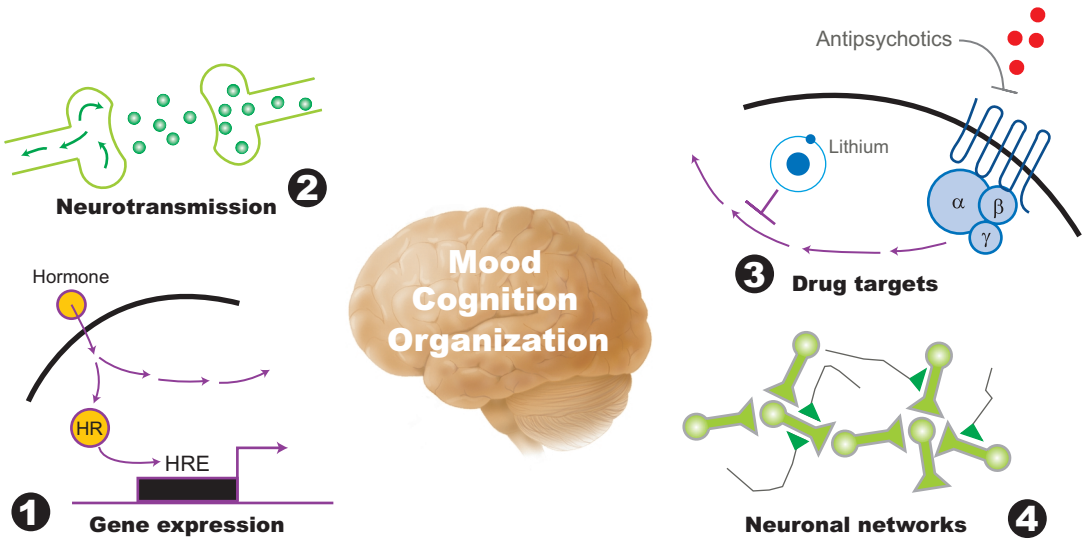

Fig. 2. Putative role of signaling pathways in the pathophysiology of psychiatric disorders. Dysfunctions of critical intracellular signaling pathways may be involved in the pathogenesis and pathophysiology of psychiatric disorders. This conclusion is based on a number of observations, including (1) the finding that many signaling pathways are modified directly or indirectly [through hormone receptors (HRs) and hormone response elements (HREs)] by neurohormones, (2) the critical role signaling pathways play in neurotransmitter and neuropeptide communication, (3) the short- and long-term cellular effects of psychiatric drugs, (4) the fact that regulation of complex signaling networks forms the basis of higher order brain function, and, in combination, the role of these processes as principal regulators of the diverse array of behavioral symptoms experienced by patients (see text for complete details).

schizophrenia phenotype already discussed, mood disorders such as bipolar disorder and depression have symptomatology that is difficult to imagine as being regulated solely at a single neurotransmitter level. For example, a single patient with bipolar disorder may be sullen, fatigued, experience thoughts of suicide, have reduced cognitive abilities, and no appetite when suffering from depression, but—at a different point in time—may experience vastly increased energy, rapid thoughts, an increased sexual drive, and may not require sleep. Even minor variations in ubiquitous regulators of signaling pathways can affect complex functions, yielding detrimental effects on behavior; this is clearly seen in many mouse models, where some genetic mutations in expressed proteins have little effect on non-CNS functions, but major effects on behavior (*15*, *37*). These effects on behavior are accomplished through many mechanisms, including the regulation of multiple neurotransmitter and neuropeptide systems, which form the basis of functional neuronal circuits. Signaling pathways are also targets for hormones that have been implicated in the pathophysiology of psychiatric illnesses including schizophrenia, such as gonadal steroids, thyroid hormones, and glucocorticoids (*18*, *38*). In toto, it is the dynamic regulation of complex, interacting neural circuits that undoubtedly forms the basis for higher order brain functions such as mood, cognition, sense of self, and reality (Fig. 2).

It is noteworthy that genetic abnormalities in signaling components are often fully compatible with life, and in many instances—despite the often-ubiquitous expression of the signaling protein—one sees circumscribed clinical manifestations. For example, a number of neurological disorders are caused by known intracellular signaling abnormalities. These include cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (*39*) (CADASIL, which results from mutations in Notch 3 and is characterized by migraines, white matter hyperintensities, and cognitive decline beginning at early mid-age) and Lafora's disease (*40*) [which results from mutations in EPM2A (epilepsy, progressive myoclonus type 2A), a tyrosine phosphatase, and is characterized by progressive myoclonus epilepsy]. Even some forms of mental retardation (*41*) [associated with mutations in p21 activated protein kinase 3 (PAK3), a serine-threonine kinase] and certain forms of early-onset Alzheimer's disease [involving mutations in presenilin I and II that, among other things, modulate Notch and β-catenin function; see (*42*) for a review] have been associated with abnormalities in intracellular signaling pathways.

These overt, yet relatively circumscribed, clinical manifestations are believed to ultimately arise from vastly different transcriptomes (all of the transcripts present at a particular time) in different tissues because of tissue-specific expression, haploinsufficiency, genetic imprinting, alternate splicing, varying stoichiometries of the relevant signaling partners in different tissues, and differences in the ability of diverse cell types to compensate for the abnormality (either autocrine or paracrine) (*8*, *43*).

Schizophrenia and cellular signaling: Recent evidence implicating calcineurin

Although different disease genes may contribute in varying degrees to schizophrenia's complex clinical presentation, the potential involvement of a molecule known to regulate multiple signaling cascades, and specifically those neurotransmitter systems most strongly implicated in schizophrenia, is quite attractive. Evidence from knockout mouse behavioral studies and human genetic association studies has recently implicated calcineurin (protein phosphatase 2B) as a schizophrenia susceptibility gene (*3*, *4*). Calcineurin is a major calmodulin-binding

protein in brain and the only known serine/threonine protein phosphatase that is directly regulated by calcium and calmodulin, which make it a potentially powerful mediator of intracellular signals. Although calcineurin has a ubiquitous distribution, it is highly enriched in neural tissue, making up a large portion of the total protein in brain. A heterodimer, calcineurin is composed of two catalytic (calcineurin A, CaNA) and two regulatory (CaNB) subunits. There are three mammalian (α, β, and γ) calcineurin catalytic isoforms and two regulatory isoforms (CaNB1 and CaNB2). Calcineurin has a number of substrates, including dynamin, nitric oxide synthase, Elk-1, dopamine and cAMP-regulated phosphoprotein of 32 kD (DARPP-32), tau, and heat shock protein 25, among many others [see (*44*-*46*) for reviews]. In addition to being regulated by intracellular calcium concentration and calmodulin, calcineurin is regulated by binding proteins, including CAIN (calcineurin inhibitor) (*47*, *48*), FKBP12 (FK-506 binding protein) (*49*), and AKAP79 (protein kinase A anchoring protein 79) (*50*).

The mouse behavioral studies implicating calcineurin in schizophrenia were based on specific analogues to schizophrenia endophenotypes. As shown in Fig. 1, endophenotypes represent neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological findings in psychiatric disease that are believed to have more simple genetic underpinnings than the complex behavioral disorders themselves [see (*12*) for a review]. Thus, because of the difficulty in objectively defining schizophrenia traits, endophenotypes are used to aid in genetic linkage and association studies, future diagnostic refinements, and the development and analysis of animal models (*12*). Tonegawa and colleagues had previously shown that knockout mice lacking CaNB1 in the forebrain have deficits in working memory (*51*), a well-documented schizophrenia endophenotype [see (*14*, *52*) for reviews]. In this most recent paper, they follow up on these original findings, showing that, in addition to working memory deficits, the mice have alterations in other schizophrenia-implicated endophenotypes, including diminished prepulse and latent inhibition (which represent measures of altered informational processing), and an increased response to the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK801 (*4*). As pointed out in their paper, these findings in the CaNB1 forebrain-specific knockout mice are strikingly similar to observations in patients with schizophrenia (*12*).

On the basis of these exciting findings, the same research group, collaborating with Maria Karayiorgou and colleagues, performed association studies with calcineurin-related genes, prioritizing those that mapped to previously identified schizophrenia susceptibility loci (*3*). They utilized linkage disequilibrium (LD) analysis of family samples (trios) that test for preferential transmission of variants to affected individuals from their unaffected parents. Within a U.S. sample of trios, single nucleotide polymorphisms (SNPs) and associated haplotypes identified allelic variants of PPP3CC [which encodes CaNAγ, a catalytic subunit, found at susceptibility loci 8p21 (Fig. 1)] that were preferentially transmitted to schizophrenic patients. By further combining their trios with a South African sample, they found an even greater effect. The association data have now been replicated within Japanese family samples in collaboration with Kazuo Yamada and Takeo Yoshikawa of RIKEN (*53*). Thus, in addition to the mouse studies, these results implicate PPP3CC (and by association calcineurin) as a schizophrenia risk gene. Because the SNPs are noncoding and of unclear functional relevance, it will be critical to identify the actual causal polymorphisms and, as with all association studies, equally essential to validate these findings in further independent samples. Future experiments will also delineate more clearly how specific functional polymorphisms may interact with environmental and epigenetic factors to mediate facets of schizophrenia pathophysiology.

Although our understanding of the specific molecular and cellular abnormalities underlying schizophrenia is in its infancy, the available data suggest that many of the acute signs and symptoms of the disease are modulated—at least in part—by dopaminergic and glutamatergic signaling, whereas the chronic course may be mediated by dysregulation of neuronal development, neuron maturation, and functional plasticity (*1*, *54*). Evidence supporting abnormalities of the dopaminergic system includes the facts that all effective antipsychotic agents block dopamine D2 receptors and that amphetamine, which facilitates dopamine release, induces schizophrenia-like psychotic symptoms. In addition, some postmortem brain and PET radioligand studies have found abnormalities of the dopaminergic system in schizophrenia [see (*55*) for a review]. It would, however, be an incorrect oversimplification to suggest that schizophrenia is simply a disease of dopamine excess, and the current data support a model of prefrontal cortical dopamine hypofunction (mainly D1 receptor mediated) accompanied by limbic dopaminergic hyperfunctioning (mediated primarily through D2 receptors). With respect to the glutamatergic system, antagonists of the NMDA receptor [such as ketamine and phencyclidine (PCP)] often induce symptoms of schizophrenia in control subjects, or they produce an exacerbation of the disease itself in remitted schizophrenia patients. By contrast, agents that facilitate NMDA receptor activity, such as D-serine and glycine, have shown efficacy in the treatment of some schizophrenic symptoms [see (*56*) for an excellent review].

Intriguingly, calcineurin, through its protein-protein interactions and its phosphatase activity, is uniquely positioned to regulate both the impairments of plasticity and dopaminergic and glutamatergic dysfunction observed in schizophrenia (Fig. 3). Calcineurin is now well established as providing a critical link between calcium regulation, synaptic plasticity, cell survival, and cognition. These findings have possible relevance to schizophrenia, where neuroimaging and postmortem studies have identified structural and cellular alterations suggesting neuronal atrophy [see (*57*-*60*) for salient examples], and neuropsychological testing has consistently identified specific memory deficits [see (*14*, *52*) for reviews]. Activation of calcineurin following excessive increases in calcium appears to promote neuronal death through dephosphorylation of two key cell survival/cell death proteins, namely, cAMP response element-binding protein (CREB) (*61*), and the Bcl-2 family member BAD (*62*). Most recently, calcineurin has been demonstrated to play an important role in the developmental regulation of axon growth (*63*). Thus, it now appears that—in addition to their direct actions on growth cone tips—neurotrophins and netrins activate a calcineurin-dependent transcriptional encode that is required for efficient embryonic axon outgrowth. Calcineurin also appears to be a direct regulator of the NMDA receptor (Fig. 3), thereby altering long-term potentiation (LTP) and long-term depression (LTD) of evoked postsynaptic current amplitude in neurons. Activating calcineurin results in function-

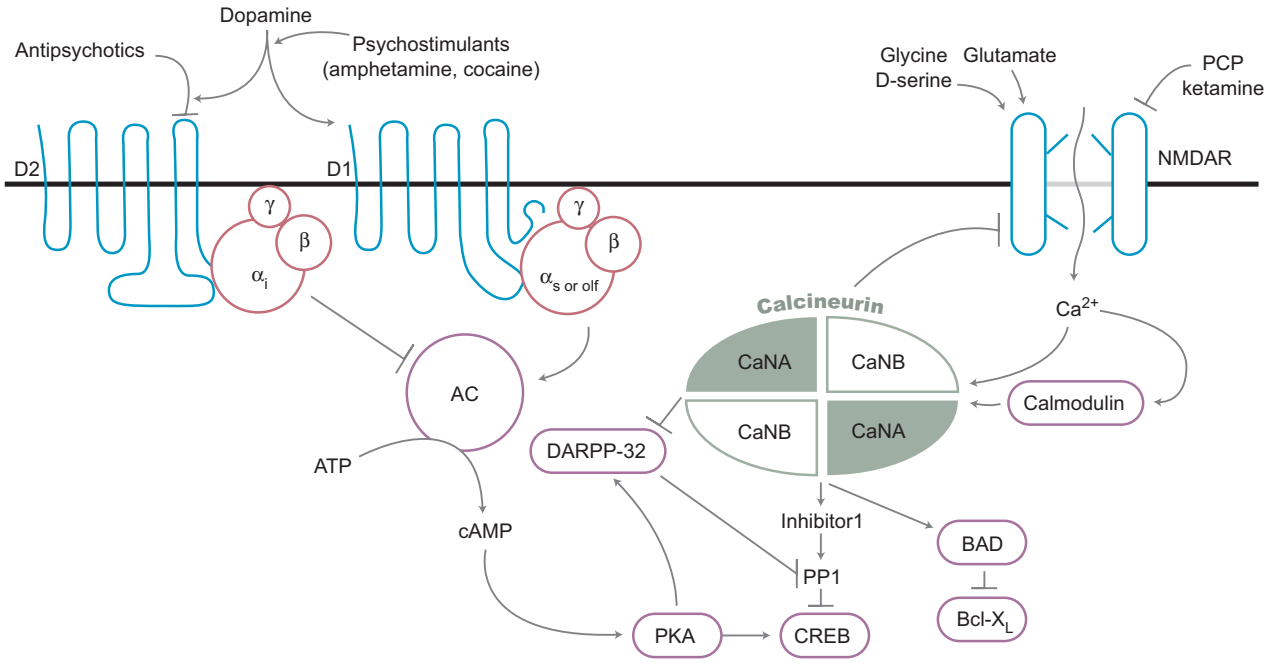

Fig. 3. Major roles of calcineurin in dopamine and NMDA signaling pathways. Dopaminergic and glutamatergic are two primary neurotransmitter systems implicated in schizophrenia. Psychostimulants (such as cocaine and amphetamine), which increase dopamine release, and NMDA antagonists (including PCP and ketamine) are psychotomimetics. D2 antagonists and NMDA agonists are effective antipsychotics. As shown in the figure, both glutamatergic and dopaminergic systems interact with calcineurin. A major source of calcium fluctuation in the cell is through the opening and closing of NMDA receptors. Calcium activates calcineurin both directly and through interactions with calmodulin. Calcineurin activation results in functional desensitization of postsynaptic NMDA calcium channels and regulates adenylate cyclase (AC)-protein kinase A (PKA)-cyclic AMP response element binding protein (CREB) signaling through interactions with DARPP-32 and inhibitor 1 (through protein phosphatase 1; PP1) (*69*). Recent evidence also suggests that calcineurin may be a critical regulator of apoptosis through interactions with Bcl-2 family member BAD (*62*), which may be of relevance to some of the cellular and structural abnormalities observed in brains of patients with schizophrenia (see text for further details).

al modulation of various glutamate receptor subtypes [see (*64*) for review], which includes the desensitization of postsynaptic NMDA receptors (*65*). Furthermore, overexpression of calcineurin in young adult animals leads to altered synaptic function and memory retention deficits (*66*).

Although calcineurin has diverse substrates that could potentially regulate dopaminergic and glutamatergic signaling, DARPP-32 may be pivotal. DARPP-32 is specifically localized to neurons containing dopamine receptors and is a potent inhibitor of protein phosphatase 1, which plays a central role in dopaminergic and glutamatergic signaling and in integrating the activity of these two pathways (*67*, *68*) (Fig. 3). Dopamine, acting through the D1 receptor and the adenylate cyclase-stimulatory G protein, G_s (and possibly G_{olf}), activates adenylate cyclase, thereby bringing about protein kinase A (PKA)-mediated phosphorylation of DARPP-32. By contrast, acting through the D2 receptor and the adenylate cyclase-inhibitory G_i proteins, dopamine reduces DARPP-32 phosphorylation. Calcineurin acts as a principal mediator of dephosphorylation-dependent inactivation of DARPP-32 [see (*69*) for a review]. As we discussed above, both subtypes of dopamine receptors have been implicated in the pathophysiology of schizophrenia. Glutamate also promotes dephosphorylation of DARPP-32, probably through calcium-dependent activation of calcineurin (*69*, *70*). Consistent with these observations, mice generated to contain a targeted disruption of the DARPP-32 gene show deficits in their molecular, electrophysiological, and behavioral responses to dopamine, drugs of abuse, and antipsychotic medications (*69*, *71*-*73*). Interestingly, the calcineurin inhibitor FK506 has recently been shown to modulate methamphetamine-induced behavioral changes in rats (*74*), and a preliminary postmortem human brain study reported reduced levels of DARPP-32 in dorsolateral prefrontal cortex in schizophrenia (*74*). Thus, calcineurin is in a unique position to modulate both dopaminergic and glutamatergic signaling (Fig. 3).

Conclusions

Psychiatric illnesses such as schizophrenia affect a large percentage of the population and are associated with immense societal and individual health care costs, loss of productivity-related unemployment and disability expenses, and medical comorbidities (*75*, *76*). Until recently, clear and reproducible evidence for the association of schizophrenia with specific genes (or their allelic variations) has been lacking. However, recent findings have implicated a number of gene regions and specific genes, including neuregulin-1, dysbindin, catechol-*O*-methyltransferase, G72, RGS4, and now PPP3CC, among others (*5*, *77*) (see Fig. 1), in the pathogenesis of schizophrenia. It is likely that genes lending susceptibility to the development of other psychiatric disorders (especially bipolar disorder) will al-

so be identified (reproducible) soon. Now the more daunting task—to discern the functional relevance of these modulations—can begin. It is likely that many of these functions will involve direct and indirect modulation of critical intracellular signaling pathways.

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