Review

Biological Psychiatry

Integrating the Neurodevelopmental and Dopamine Hypotheses of Schizophrenia and the Role of Cortical Excitation-Inhibition Balance

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ABSTRACT

The neurodevelopmental and dopamine hypotheses are leading theories of the pathoetiology of schizophrenia, but they were developed in isolation. However, since they were originally proposed, there have been considerable advances in our understanding of the normal neurodevelopmental refinement of synapses and cortical excitation-inhibition (E/I) balance, as well as preclinical findings on the interrelationship between cortical and subcortical systems and new in vivo imaging and induced pluripotent stem cell evidence for lower synaptic density markers in patients with schizophrenia. Genetic advances show that schizophrenia is associated with variants linked to genes affecting GABA (gamma-aminobutyric acid) and glutamatergic signaling as well as neurodevelopmental processes. Moreover, in vivo studies on the effects of stress, particularly during later development, show that it leads to synaptic elimination. We review these lines of evidence as well as in vivo evidence for altered cortical E/I balance and dopaminergic dysfunction in schizophrenia. We discuss mechanisms through which frontal cortex circuitry may regulate striatal dopamine and consider how frontal E/I imbalance may cause dopaminergic dysregulation to result in psychotic symptoms.

This integrated neurodevelopmental and dopamine hypothesis suggests that overpruning of synapses, potentially including glutamatergic inputs onto frontal cortical interneurons, disrupts the E/I balance and thus underlies cognitive and negative symptoms. It could also lead to disinhibition of excitatory projections from the frontal cortex and possibly other regions that regulate mesostriatal dopamine neurons, resulting in dopamine dysregulation and psychotic symptoms. Together, this explains a number of aspects of the epidemiology and clinical presentation of schizophrenia and identifies new targets for treatment and prevention.

https://doi.org/10.1016/j.biopsych.2022.06.017

Schizophrenia is a common and disabling mental illness that is associated with psychotic symptoms, negative symptoms, and cognitive symptoms, such as impairments in executive function and working memory (1). Two key hypotheses for schizophrenia pathoetiology are the dopamine hypothesis (2) and the neurodevelopmental hypothesis (3,4). The latter has recently been reframed as a sociodevelopmental hypothesis to account for the key role that psychosocial factors play in the developmental processes underlying schizophrenia (5). These lines of thought were initially developed largely in isolation. However, recent evidence of altered excitation-inhibition (E/I) balance in schizophrenia, studies modeling synaptic pruning mechanisms, genome-wide association studies (GWASs), and novel imaging techniques localizing synaptic markers have all shown how these hypotheses may be integrated with previous work on E/I balance (6–8). Here, we first review normal synaptic development and evidence for neurodevelopmental abnormalities in schizophrenia before considering the evidence for E/I imbalance in schizophrenia, and then propose a new integrative hypothesis of schizophrenia that ties together the dopamine and neuro(socio)developmental theories of the disorder.

SYNAPTIC DYNAMICS DURING NEURODEVELOPMENT

Studies conducted with rodents and nonhuman primates have shown that synaptic density in the brain shows marked increases early in development, followed by a period of synaptic elimination from puberty into early adulthood and then relatively stable synaptic density (9–13). Importantly, these developmental stages occur at different time points for different brain regions in a caudo-rostral manner, with the somatosensory and visual regions among the first to reach synaptic stability and the frontal cortex developing last (14).

Human postmortem brain samples assessed by electron microscopy (15,16) show the same temporal pattern, with peak synaptic density in the frontal cortex in early childhood followed by a gradual decline into the third decade of life (16). Work comparing samples from the middle frontal gyrus to Heschl's gyrus (auditory cortex) showed that developmental

ISSN: 0006-3223

Biological Psychiatry September 15, 2022; 92:501–513 www.sobp.org/journal

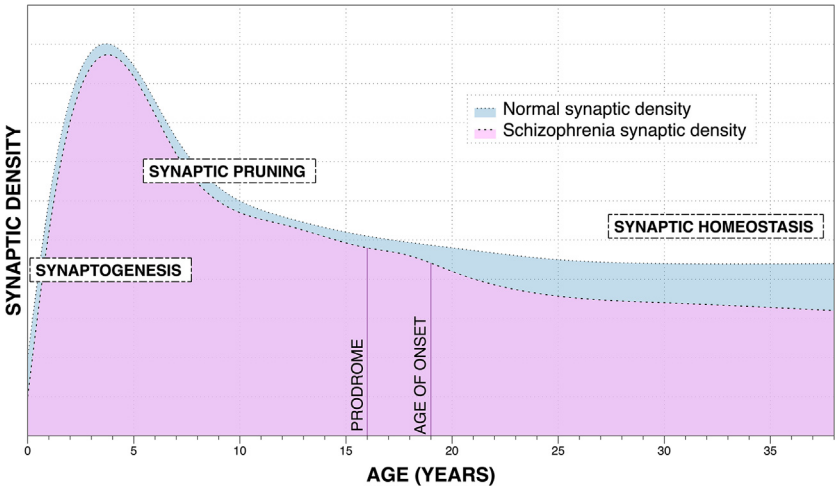

Figure 1. Synaptic trajectories during normal neurodevelopment show a period of net synaptic production throughout early childhood followed by net synaptic elimination during adolescence and early adulthood, and then relatively balanced synaptic elimination and production in middle age. In schizophrenia, induced pluripotent stem cells show a failure to form as many synapses as seen in control lines early in development (equivalent to the prenatal stage). Imaging studies also report progressive gray matter volume changes in the prodrome and early phase of illness. Based on these findings, we propose that there is also aberrant synaptic formation/pruning both early and later in neurodevelopment, leading to overpruning of synapses and excitatory/inhibitory imbalance in schizophrenia. Further patient studies are required to determine the course of synaptic loss.

trajectories are heterochronous across regions, with frontal regions maturing later than posterior regions, similar to rodent and primate research (17). In line with this, synaptic developmental trajectories of the human visual cortex (V1) have been directly aligned with the V1 of rodents, with synaptic protein expression data suggesting that development continues into late childhood (18).

Structural magnetic resonance imaging (MRI) studies provide proxy markers that could reflect changes in synaptic density. Cortical thickness and gray matter volumes increase rapidly during childhood followed by reductions during puberty and early adolescence (19,20). Importantly, different brain regions differ in when gray matter markers reach their peak, start to fall, and then stabilize, with higher-order association areas such as the dorsolateral prefrontal cortex (PFC) maturing later than sensory areas (19,21,22), thus showing the same pattern of tempororegional structural changes seen in preclinical research (20,23) and human postmortem studies of synaptic measures (summarized in Figure 1) (17).

IMAGING EVIDENCE FOR ABERRANT NEURODEVELOPMENT IN SCHIZOPHRENIA

Early brain development can be studied in vivo in patients using MRI techniques that measure the gyrification index, a metric that quantifies the amount of cortex buried within the sulcal fold. Formation of gyri during early brain development underlies compact wiring (24) and is reflected in a higher gyrification index in adulthood, which has been shown to be lower in patients with schizophrenia than in control subjects (25). Specifically, patients with schizophrenia have been reported to have reduced folding of the anterior cingulate cortex (25,26) and other alterations suggesting impaired gyral formation in the frontal cortex (27,28). As the gyrification index is determined during early development and remains stable in adulthood (24), these findings likely reflect early developmental abnormalities.

Schizophrenia is also associated with lower gray matter volumes relative to control subjects, in particular in the frontal cortex, (29,30). The progressive loss of gray matter exceeding normal age-related changes in schizophrenia indicates a neuroprogressive process, albeit one that does not result in neuronal death (31,32). Gray matter reduction in the absence of neuronal loss is consistent with the loss of synapses, but it is important to recognize that other changes could contribute to gray matter changes in schizophrenia, such as reduced neuronal processes and branching (33). Further analyses found that greater gray matter loss was directly associated with greater duration of illness (34,35), suggesting that there is at least a component of gray matter changes that occurs once the illness has developed. A number of longitudinal studies have tested this further by measuring changes in gray matter volumes over the course of illness from the first episode of psychosis (36). These studies have found that patients with schizophrenia show accelerated reductions in gray matter volumes in comparison to both their healthy siblings (37) and matched healthy control subjects (37–39). One issue with these findings is the potential role of antipsychotic treatment on gray matter changes. However, follow-up of patients that start treatment suggests that, while medication may make some contribution to gray matter reductions, an appreciable component of gray matter change is not explained by treatment (40,41).

Thus, taken together, the gyrification and gray matter findings suggest that schizophrenia is associated with both early and late disruption in neurodevelopment, including progressive changes during the early phase of the disorder. However, these MRI studies did not directly measure synaptic markers, so the degree to which they reflect synaptic loss or other changes in neuropil remains unclear.

EVIDENCE FOR ABERRANT SYNAPTIC DENSITY IN SCHIZOPHRENIA

Postmortem studies have investigated synaptic protein levels as well as dendritic spine densities in schizophrenia. Synaptophysin, a vesicular protein that is a widely used in vitro marker of synaptic density, has been shown to be significantly

lower at the protein and messenger RNA levels in postmortem samples from patients with schizophrenia relative to healthy control subjects, specifically in the hippocampus and frontal and cingulate cortices (42). Another recent meta-analysis looking at postsynaptic density markers also identified reductions in synaptic markers in frontal regions in patients with schizophrenia relative to control subjects (43).

Further evidence comes from in vivo work, using [^{11}C]UCB-J PET imaging, which measures the distribution of synaptic vesicle protein 2A (SV2A). SV2A is a ubiquitously expressed synaptic vesicle protein, and thus differences in protein levels can reflect differences in synaptic density (44,45). To date, 2 studies have been published comparing chronic patients with schizophrenia with control subjects. Both studies showed significantly lower SV2A density in frontal and anterior cingulate cortices in the patient groups (46,47). These and the postmortem studies thus provide evidence for a failure to form synapses and/or loss of synapses in the frontal cortex of patients with schizophrenia and potentially in other brain regions. Moreover, further analyses have shown that there is an altered relationship between SV2A and glutamate levels in patients with schizophrenia (48). Research using induced pluripotent stem cells (iPSCs) has shown reduced neuronal branching and impaired synaptic formation and increased engulfment of glutamatergic synaptosomes by microglia when the cells were cultured from patients with schizophrenia compared with those cultured from matched control subjects. (49–51) [for further details see (52)]. However, it is important to note that while the data to date are consistent with a failure to form synapses and/or greater synaptic elimination, it remains to be established whether both processes or just one occurs in patients.

These postmortem and in vivo lines of evidence indicate that altered synaptic elimination in the frontal cortex may affect excitatory (glutamatergic) synapses. However, as GABA (gamma-aminobutyric acid) was not measured in the in vivo study, further work is required to determine whether inhibitory terminals are also affected and, if so, how this compares to glutamatergic effects in vivo. In view of this, we now consider E/I balance and how it may be altered in schizophrenia.

E/I BALANCE

E/I balance refers to the relative contribution of excitatory and inhibitory synaptic inputs to brain signaling (53). The integration of these inputs is required for effective information processing carried out by the brain and occurs at the level of individual neurons, localized neuronal circuits, and whole-brain networks. During neurodevelopment, significant shifts in E/I balance occur during a critical period for each brain region when the region is most susceptible to inputs governed by environmental factors (54,55). The critical periods for different regions occur in a caudo-rostral manner, following a similar trajectory to synaptic markers during brain development described previously, with the frontal cortex maturing last (55,56). During this time, key mechanisms are upregulated to prevent runaway signaling while achieving a high cortical signal-to-noise ratio (53). These mechanisms include adaptation of synaptic efficacy, membrane excitability, and synapse number (53). In particular, synaptic modification, such as pruning of excitatory synapses to increase inhibitory activity, helps prevent neural activity from back-propagating through the cell body into the dendritic tree and leading to unwanted activity (55). Paolicelli *et al.* (57) have shown that synaptic elimination is facilitated by microglia. One mechanism through which this occurs is synapses expressing a molecular tag that recruits complement proteins, identifying them as targets for engulfment by microglia (58). Mice lacking complement cascade components exhibit enhanced excitatory synaptic connectivity in the mature cortex as a result of inhibited synaptic pruning (59), while mice overexpressing complement factor 4A (C4A) have increased synaptic engulfment by glia, reduced cortical synaptic density, and altered behavior (60).

GENETIC RISK AND EXCITATORY AND INHIBITORY NEUROTRANSMISSION IN SCHIZOPHRENIA

GWASs have shown that schizophrenia is a polygenic disorder, with multiple low-penetrance variants contributing to the genetic risk for the disorder (1). One of the most significant genetic associations with schizophrenia implicates genes of the major histocompatibility locus encoding adaptive immune system components. This arises in part from the presence of many structurally diverse alleles of a complement protein, C4A, which tags synapses for elimination by microglia (61). In addition, several other genes with roles in microglia-mediated pruning have been identified in GWASs (Table 1). Many of the other loci associated with schizophrenia encode excitatory and inhibitory neurotransmission components or play a role in establishing E/I balance during neurodevelopment as summarized in Table 1 and with further detail in Table S1.

Key loci associated with schizophrenia risk linked to excitatory neurotransmission include components of the NMDA receptor (NMDAR) (subunit 2A), the AMPA receptor (glutamate receptor 1), and the metabotropic glutamate receptor 3 (*GRM3*) genes (62). They also include loci encoding channel components affecting membrane excitability, enzyme serine racemase, which catalyzes synthesis of the glutamate co-agonist D-serine, as well as genes encoding components of the postsynaptic protein scaffold of excitatory synapses including postsynaptic density protein 93 (PSD-93) and SYNGAP1, which is thought to be involved in NMDAR-dependent control of AMPA receptor potentiation (62,63).

Schizophrenia-associated loci encoding proteins involved in inhibitory neurotransmission include $GABA_B$ receptor components GABBR1 and GABBR2 (62,64) and loci linked to proteins that mediate GABA receptor turnover such as ankyrin-G (ANK3), which promotes stability of somatodendritic GABAergic synapses (62,65,66). Furin, a protein involved in GABAergic transmission, also influences expression of $GABA_A$ receptor components and has been implicated in schizophrenia GWASs along with *CLCN3* and *SLC32A1* (encoding the vesicular GABA transporter), both of which are involved in controlling GABA uptake into synaptic vesicles (62,65,67,68).

These findings, summarized in Figure 2, indicate that genetic risk for schizophrenia affects proteins involved in both excitatory and inhibitory signaling, which together could predispose an individual to E/I imbalance, although the direction of the imbalance cannot be inferred based on genetic data alone. This imbalance could occur through effects on

Table 1. Loci Associated With Schizophrenia Identified by Genome-wide Association Studies (GWASs) That Have a Functional Role in Excitatory and Inhibitory Signaling or Synaptic Pruning

Gene	Protein and Functional Role
Genes for Proteins Involved in Excitatory Neurotransmission	
ADAM10	ADAM metallopeptidase domain 10 (ADAM10) is a metalloprotease involved upstream of the pathway leading to synapse elimination by microglia. It is trafficked and is functional at the excitatory synapse membrane.
AKT3	AKT serine/threonine kinase 3, AKT activity shown to inhibit metabotropic glutamate receptor (mGluR) mediated long-term depression, plays a role in synaptic plasticity in the hippocampus
CACNA1	Pore-forming, alpha-1C subunit of the voltage-gated calcium channel that gives rise to L-type calcium currents
CACNA1D	L-type voltage-gated calcium channel α-1D subunit
CACNA1	Calcium voltage-gated channel subunit alpha1 I, T-type calcium channel subunit, involved in neuronal calcium signaling
CACNB2	Voltage-dependent L-type calcium channel subunit beta-2, component of a calcium channel complex, involved in neuronal calcium signaling
DLG2	Discs large MAGUK scaffold protein 2 (DLG2) is part of the postsynaptic protein scaffold of excitatory synapses and is involved in NMDA signaling.
FLOT1	Flotillin-1 (FLOT1) enhances the formation of glutamatergic synapses but not GABAergic synapses. Flot1 has been shown to be essential for amphetamine-induced reverse transport of DA in neurons but not for DA uptake.
GRIA1	Glutamate ionotropic receptor AMPA type subunit 1
GRIN2A	Glutamate ionotropic receptor NMDA type subunit 2A
GRM3	Glutamate metabotropic receptor 3
HCN1	The hyperpolarization-activated cyclic nucleotide-gated (HCN1) channels modulate the rate of glutamate release by changing rate of exocytosis in synaptic terminals.
RYR3	Ryanodine receptor type 3 (RyR3) involved in Ca signaling
SRR	Serine racemase catalyzes the synthesis of D-serine from L-serine. D-serine is a key coagonist with glutamate at NMDA receptors.
SYNGAP1	Synaptic ras GTPase activating protein 1 (SYNGAP1) is a member of the NMDAR signaling complex in excitatory synapses and may play a role in NMDAR-dependent control of AMPAR potentiation, AMPAR membrane trafficking, and synaptic plasticity.
Genes for Proteins Involved in Inhibitory Neurotransmission	
ANK3	Ankyrin-G/ankyrin-3 (ANK3) is integral to AMPAR-mediated synaptic transmission and maintenance of spine morphology. It promotes stability of somatodendritic GABAergic synapses in vitro and in vivo through opposing endocytosis of $GABA_A$ receptors.
CLCN3	Chloride voltage-gated channel 3 plays a role in inhibitory transmission via neurotransmitter loading of synaptic vesicles dependent on vesicular acidification. Cl^- in inhibitory transmission may be both postsynaptic permeant species and a presynaptic regulatory element.
FURIN	Furin, a protease enzyme, is involved in $GABA_A$-mediated synaptic transmission.
GABBR1	γ-aminobutyric acid type B receptor subunit 1
GABBR2	γ-aminobutyric acid type B receptor subunit 2
PLCL1	Phospholipase C like 1 regulates the turnover of $GABA_A$ receptors via phospho-dependent endocytosis and thus contributes to the maintenance of GABA-mediated synaptic inhibition.
SLC32A1	Solute carrier family 32 member 1 is involved in the uptake of GABA and glycine into the synaptic vesicles.
Microglial Genes With a Known Function in Synaptic Pruning	
ADAM10	ADAM metallopeptidase domain 10 (ADAM10) is a metalloprotease involved upstream of the pathway leading to synapse elimination by microglia. It is trafficked and is functional at the excitatory synapse membrane.
CSMD1	Regulator of C4 expression
C4	Complement component 4, protein expressed on synapses to tag them for elimination by microglia
PDE4B	Phosphodiesterase 4B is a microglia target to reduce neuroinflammation, also expressed at the synapse.
VRK2	Vaccinia-related kinase 2 plays a critical role in microglia-mediated synapse elimination during neurodevelopment.
Genes for Proteins Involved in Establishing E/I Balance During Neurodevelopment	
AMBRA1	Autophagy and beclin 1 regulator 1 (Ambra1) is implicated in neurodevelopment, playing a key role in the maturation of hippocampal parvalbumin interneurons and thus in maintaining a proper excitation/inhibition balance in the brain.
CLSTN3	Calsyntenin-3 promotes inhibitory and excitatory synaptic development.
CUL3	Culin-3 is compartmentalized at postsynaptic densities and gates retrograde signaling; it is involved in neural development, neurotransmission, and maintaining E/I balance and glutamate receptor turnover.
FOXP1	Forkhead box protein 1 is a transcription factor for genes associated with synaptic function and development.
GPM6A	Glycoprotein M6A contributes to spine and, likely, synapse formation.
HIP1R	Huntingtin-interacting protein 1-related protein plays a critical role in dendritic development and excitatory synapse formation in hippocampal neurons.

Table 1. Continued

Gene	Protein and Functional Role
IGSF9B	Immunoglobulin superfamily member 9B is a transmembrane protein which is abundantly expressed in interneurons, where it may regulate inhibitory synapse development.
KALRN	Kalirin7 is involved in the formation of dendritic spines.
LRRTM4	Leucine rich repeat transmembrane neuronal 4 is involved in regulating excitatory synapse development.
MEF2C	Myocyte enhancer factor 2C plays a role in hippocampal-dependent learning and memory by suppressing the number of excitatory synapses and thus regulating basal and evoked synaptic transmission. Crucial for normal neuronal development, distribution, and electrical activity in the neocortex
NLGN4X	Neuroligin 4 X-linked is a member of the neuroligin family of proteins, which are involved in the regulation of excitatory synaptic transmission.

AMPAR, AMPA receptor; DA, dopamine; E/I, excitation-inhibition; GABA, gamma-aminobutyric acid; NMDAR, NMDA receptor.

homeostatic synaptic scaling or during initial circuit formation, given that risk loci encoding neurodevelopmental genes contributing to E/I balance during circuit formation have also been identified (Table 1). One caveat is that many variants associated with schizophrenia also occur outside coding regions (69). Their effects and those of other risk variants on E/I balance remain to be investigated. Key future experiments include iPSC models, where a variant can be knocked down in the presence of a schizophrenia genetic background, or animal models similar to those that have clarified the genetic effects of high-penetrance variants such as the 22q11.2 deletion on E/I balance in schizophrenia (70). Importantly, effects need to be considered at the systems level because they may vary by circuit and depend on the state of the rest of the system. In view of this, we next review in vivo evidence for E/I imbalance at the whole-brain level in patients with schizophrenia.

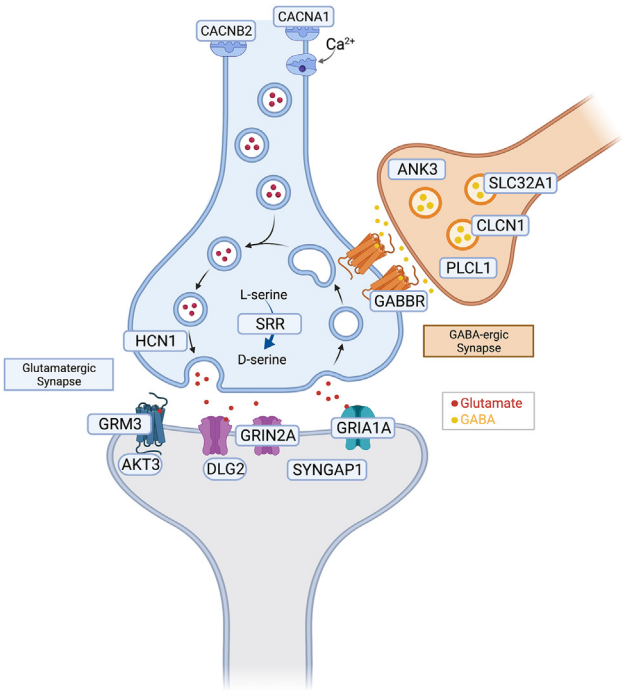

Figure 2. Genes encoding inhibitory and excitatory signaling components identified by schizophrenia genome-wide association studies associated with schizophrenia risk. AKT3, AKT serine/threonine kinase 3; ANK3, ankyrin-G/ankyrin-3; CACNA1, voltage-gated calcium channel subunit alpha1; CACNB2, voltage-dependent L-type calcium channel subunit beta-2; CLCN3, chloride voltage-gated channel 3; DLG2, discs large MAGUK scaffold protein 2; GABBR, γ-aminobutyric acid type B receptor; GRIN2A, glutamate ionotropic receptor NMDA type subunit 2A; GRIA1, glutamate ionotropic receptor AMPA type subunit 1; GRM3, glutamate metabotropic receptor 3; HCN1, hyperpolarization-activated cyclic nucleotide-gated channel component; PLCL1, phospholipase C like 1; SLC32A1, solute carrier family 32 member 1; SRR, serine racemase; SYNGAP1, synaptic Ras GTPase activating protein 1.

IN VIVO EVIDENCE FOR ALTERED E/I BALANCE IN SCHIZOPHRENIA

Electroencephalography (EEG) and magnetoencephalography techniques provide measures of neural responses mediated by GABAergic and glutamatergic systems (71). Typically, patients are reported to have elevated gamma power at rest, thought to be due to impaired GABA signaling (72–74). They also have sensory gating deficits, specifically, impaired suppression of the P50 early event-related potential, which is mediated through $GABA_B$ receptors that are located on glutamatergic afferents and that inhibit pyramidal neuron firing (75–77). The combination of transcranial magnetic stimulation with EEG provides another method of probing changes in $GABA_A$, $GABA_B$, and NMDA-mediated activity using paradigms such as short-interval intracortical inhibition, long-interval intracortical inhibition, and intracortical facilitation, respectively (78) (additional details in the Supplement). These responses have been shown to be reduced in patients with schizophrenia in comparison to control subjects (79). Another measure, the mismatch negativity response, is dependent on intact NMDAR signaling (80,81). Results of a meta-analysis have shown that the mismatch negativity response is lower in patients with schizophrenia than in healthy control subjects, with a large effect size (82) and with a recent study showing that reduced mismatch negativity response amplitude was associated with reduced glutamate levels measured with magnetic resonance spectroscopy in this patient group (83). This is consistent with findings of lower NMDAR levels in schizophrenia (84). Notwithstanding this, postmortem studies show lower levels of GABAergic markers in cortical brain regions (85). While the previously mentioned studies all indicate an E/I imbalance, they do not infer the location and direction of the shift and may be confounded by the effects of medication on magnetoencephalography/EEG signal. Computational modeling of EEG data from schizophrenia patients suggests that deficits are

best explained by primary loss of synaptic gain on pyramidal cells that is then compensated by interneuron downregulation (86).

These findings are consistent with altered E/I balance in schizophrenia and have been linked with cognitive symptoms including impaired executive function (87). Both altered gamma oscillatory activity (71,88) and dorsolateral prefrontal cortical short-interval intracortical inhibition responses are correlated with cognitive function in schizophrenia (89). Recent work has also shown working memory deficits following administration of ketamine, a NMDAR antagonist, to nonhuman primates. These resembled deficits seen in schizophrenia and were accompanied by decreased inhibitory interneuron and increased excitatory activity in the lateral PFC (90). Thus, these findings indicate that E/I imbalance could underlie cognitive impairments in schizophrenia. In the following sections we consider the key question of how these cortical impairments may also lead to psychotic symptoms.

DOPAMINE ABNORMALITIES IN SCHIZOPHRENIA

Multiple lines of evidence from genetic, postmortem, and pharmacological studies support the hypothesis that dopamine dysregulation plays a central role in the development of schizophrenia (91–93). Notably, all currently licensed antipsychotics are dopamine D_2/D_3 receptor blockers (85). Moreover, molecular imaging techniques have found significant elevations in striatal dopamine synthesis and release capacity in vivo in patients with schizophrenia, with large effect sizes (94–99). Moreover, meta-analysis has shown that the largest increases are seen in parts of the striatum that are highly innervated by projections from the frontal cortex (96,100,101), and greater dopamine synthesis capacity in this region is directly associated with more severe psychotic symptoms (102,103). In contrast, striatal regions that are innervated by limbic areas show much less marked changes on average (96).

Elevated striatal dopamine synthesis and release capacity has also been found in people at genetic and/or clinical high risk for schizophrenia in some studies (100,104,105) although not in all, potentially because not all patients are actually in the prodrome to schizophrenia (106). Notwithstanding this issue, dopaminergic elevations were most marked in striatal regions innervated by frontal cortical projections, as with schizophrenia, and greater elevation here is associated with more severe prodromal-type symptoms (95,107).

EVIDENCE CORTICAL DISRUPTION LEADS TO STRIATAL DOPAMINE OVERACTIVITY

Several lines of preclinical and clinical evidence indicate that the activity of mesostriatal dopaminergic neurons is regulated by cortical projections, specifically from the frontal cortex. Lesions of the frontal cortex lead to increased striatal dopamine levels in rats (108,109). More recent work shows that applying electrical and optogenetic stimulation to the medial PFC results in striatal dopamine release both directly through excitatory afferents (110) and indirectly through further activation of cholinergic and glutamatergic systems (110,111). Evidence that synaptic changes might be involved comes from a mouse model that leads to the loss of synapses onto excitatory neurons in the frontal cortex (112). Progressive spine loss in this model led to increased striatal dopamine levels comparable to those from optogenetic simulation of circuitry connecting the frontal cortex with the ventral tegmental area/substantia nigra pars compacta (112). This study also showed that both frontal optogenetic stimulation and progressive cortical synaptic loss lead to hyperlocomotion as well as to increased striatal dopamine (112).

NMDAR antagonists such as ketamine cause negative, cognitive, and positive symptoms in healthy volunteers and worsen symptoms in patients with schizophrenia (113). Mice treated with subchronic ketamine present with hyperlocomotion, locomotor sensitization, and increased striatal dopamine synthesis capacity (114). Moreover, this effect is dependent on midbrain dopamine neuron firing and can be prevented by activating inhibitory interneurons in cortical regions, highlighting that cortical E/I balance influences subcortical dopamine neuron function (114). Subchronic ketamine administration is also associated with elevated resting gamma power (72), as seen in schizophrenia (see above). This effect of ketamine was partially rescued through tonic inhibition of the basal forebrain, further highlighting the potential role of E/I balance (115).

In healthy control subjects, a single dose of ketamine increases amphetamine-induced striatal dopamine release (116), which mimics the higher dopamine release to an amphetamine challenge in schizophrenia. Data from patient studies also show a potential link between frontal cortical measures and striatal dopamine function. For example, striatal dopamine synthesis capacity was shown to be negatively correlated with prefrontal gray matter volume in patients with schizophrenia (117). Furthermore, lower *N*-acetylaspartate levels in the dorsolateral PFC were associated with greater amphetamine-induced release of striatal dopamine in patients with schizophrenia, but not in healthy control subjects (118). As lower *N*-acetylaspartate levels are associated with neuronal dysfunction (119), this suggests that impaired frontal neuronal function is associated with elevated striatal dopamine release. Consistent with this, altered prefrontal activation during cognitive tasks testing verbal fluency and working memory has also been shown to directly relate to striatal dopamine function in schizophrenia and people at risk of psychosis (120,121). Glutamate concentration in the anterior cingulate cortex has also been shown to correlate with striatal dopamine synthesis capacity in first-episode psychosis patients but not in control subjects (122). Thus, overall, preclinical studies show that the frontal cortex regulates striatal dopamine function, and healthy volunteer challenge and patient studies show that frontal function is linked to striatal dopamine measures.

EFFECTS OF STRESS ON E/I BALANCE AND SYNAPTIC DENSITY

Rodent studies show that a range of stressors affect frontal E/I balance. Prenatal stress exposure (123), social instability stress (124), and stress during adolescence are all associated with altered excitability of the PFC and changes in E/I molecular markers (125). Acute stress has also been shown to decrease synchronous activity of both excitatory and inhibitory

neurons (126). Moreover, cortical E/I imbalance caused by stress in the adolescent period persists into adulthood along with impaired GABA and glutamate uptake into neurons (125).

Prenatal and adolescent stress exposure also result in PFC and hippocampal synaptic loss mediated by microglia (127–133). Studies investigating mechanisms of stress-related neuronal remodeling suggest that it occurs at least in part through complement-dependent synaptic elimination. Chronic stress upregulates complement C3, a molecular tag that labels synapses for deletion by microglia (134,135). Viral upregulation of C3 similarly enhances synaptic pruning, while C3 knockouts have a reduced stress response to social withdrawal (131,132). There is also evidence for altered markers of microglial activity in schizophrenia (136). Together, these findings suggest that microglia may mediate aberrant synaptic pruning that leads to E/I imbalance.

Numerous rodent studies show that effects on E/I balance and enhanced microglial pruning and resultant synaptic loss are more marked in males than females (137,138). For example, PFC E/I imbalance due to prenatal stress was shown in male but not female rodents (123). Chronic unpredictable stress causing synapse elimination by glia was also shown in males only (129,130). Thus, greater vulnerability to the effects of stress on synaptic elimination could account for findings that schizophrenia shows an earlier onset in men than in women (1).

AN INTEGRATED HYPOTHESIS

The evidence reviewed above suggests that there may be a failure to form synapses and/or greater elimination of them later in neurodevelopment in people who go on to develop schizophrenia, an effect which is at least partly mediated by genetic risk variants that dysregulate pruning of synapses by microglia. Moreover, genetic vulnerability for schizophrenia affects multiple genes involved in excitatory and inhibitory signaling. This could make circuits particularly vulnerable to tip into E/I imbalance during adolescence and early adulthood, when there is significant refinement of synapses during normal neurodevelopment.

Environmental risk factors for schizophrenia, such as psychosocial stressors, could then act on this vulnerable system. As discussed earlier, stress leads to increased glutamatergic synaptic elimination in frontal cortical regions. We propose that this leads to preferential loss of local excitatory synapses that provide feedback regulation of pyramidal neurons to tip vulnerable cortical circuits into E/I imbalance (Figure 3). This is anticipated to lead to increased noise in cortical circuits, impairing cortical function and leading to the cognitive and negative symptoms of the disorder. We propose that this also disinhibits excitatory projections that regulate mesostriatal dopamine neurons, resulting in dopamine dysregulation and psychotic symptoms through disrupting prediction error signaling [for a review see (139)]; this process is outlined in Figures 4 and 5. The late maturation of the frontal cortex, and findings that stress leads to synaptic elimination there, make it particularly vulnerable to tip into E/I imbalance, although other regions may also be affected.

The timing of these processes fits with the time course for the development of symptoms, which typically begin with cognitive impairments, and then the development of negative symptoms followed by psychotic symptoms (92).

OUTSTANDING ISSUES

We use the term E/I imbalance to highlight that it remains to be established whether it is excitatory or inhibitory changes that are causal in schizophrenia and because a change in excitation could lead to knock-on changes in inhibition and vice versa, resulting in similar disruption of cortical circuits. Thus, key questions are the precise localization of E/I imbalance within cortical circuitry, the direction of the shift in E/I at different developmental time points, and whether aberrant pruning affects specific circuits or is a global process. We have proposed that there is preferential loss of local excitatory synapses that provide feedback regulation of pyramidal neurons. However,

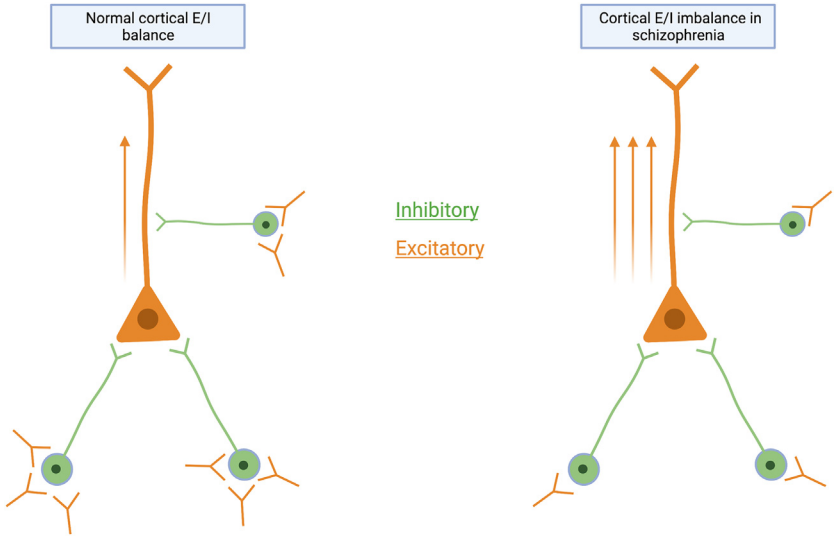

Figure 3. Aberrant E/I balance in the frontal cortex of patients with schizophrenia. Lower levels of excitatory synaptic inputs onto inhibitory interneurons (shown in green) are proposed to result in increased activity of pyramidal neurons (shown in orange, arrows indicate activity). E/I, excitation-inhibition.

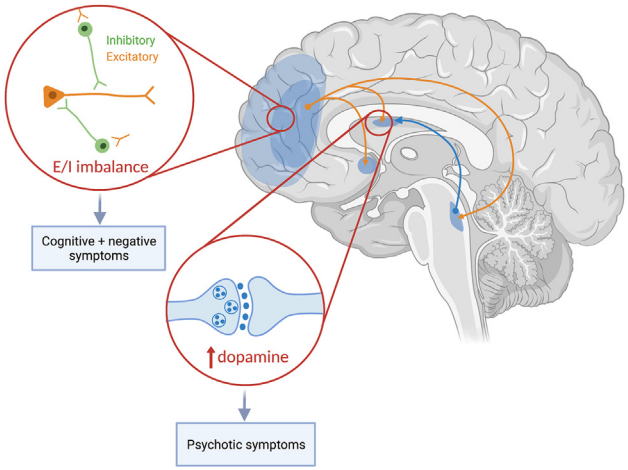

Figure 4. Projections from the frontal cortex to the striatum and midbrain origin of dopamine neurons. Frontal E/I imbalance could lead to dopamine dysfunction in schizophrenia. Orange arrows indicate cortical glutamatergic projections, blue arrow indicates dopaminergic projections from substantia nigra/ventral tegmental area to caudate. Cognitive symptoms include impairments in working memory, attention, and executive function. E/I, excitation-inhibition.

while there is some supporting evidence for this from in vivo and in vitro studies (140,141), further work is required to replicate these findings and investigate whether other glutamatergic synapses may also be lost. Given that markers of frontal E/I balance in schizophrenia differ depending on the anatomical resolution studied (142), it is important to carry out layer- and cell type–specific studies to address these issues as well as preclinical studies to determine whether loss of excitatory input onto GABAergic interneurons leads to phenotypes associated with schizophrenia. It is also unclear how aberrant pruning affects inhibitory synapses and whether changes to inhibitory signaling contribute to adaptive compensatory change or toward pathology. In addition, there is some evidence that areas other than the PFC, such as the hippocampus, are vulnerable to synaptic loss, and further work is required to map how other regions may contribute to disturbances discussed in this review.

Furthermore, while we have highlighted the potential role of C4A in schizophrenia, multiple interacting proteins in the complement systems as well as other factors that modulate complement and microglial activity are involved in synaptic pruning (143). It remains to be determined whether and how these contribute to a vulnerability to aberrant synaptic pruning in schizophrenia.

We have also proposed that there is impaired synaptic formation early in neurodevelopment in schizophrenia. While there is less evidence for this, iPSC studies modeling circuit formation may be useful to better model this developmental stage in schizophrenia. It should also be recognized that synaptic plasticity, and not just absolute synaptic density, is important to cognitive development (144).

One final issue is that while, as we have highlighted, there are data showing that frontal and striatal dopamine function are related in schizophrenia, the causal relationship we propose has not been directly tested in patients. This requires longitudinal studies to investigate whether aberrant pruning and E/I imbalance lead to striatal hyperactivity via PFC dysfunction and whether overpruning in schizophrenia may continue into adulthood.

Finally, stress is a risk factor for many other psychiatric disorders, but why does it lead to schizophrenia in some people and other presentations in others? The answer likely lies in the individual's other vulnerability factors, particularly genetic variants, which influence the circuits that are vulnerable to the effects of stress on synaptic pruning. Studies investigating the interactions between these factors and the effects of stress would help address this issue. It should also be recognized that, while the genetic variants implicating synaptic alterations in schizophrenia that we have discussed are significant at the genome-wide level, it remains unclear how prevalent they are across cases. Similarly, some other variants associated with schizophrenia do not currently implicate synaptic alterations, and some patients do not show the dopaminergic alterations seen in the majority (103,145). Thus, other mechanisms may underlie symptoms in these patients, consistent with the idea that there are neurobiological subtypes in schizophrenia (146).

IMPLICATIONS FOR TREATING SCHIZOPHRENIA

Targeting E/I imbalance may be a novel approach to treating cognitive and negative symptoms of schizophrenia. There are a number of potentially procognitive compounds in development that could do this such as modulators of inhibitory

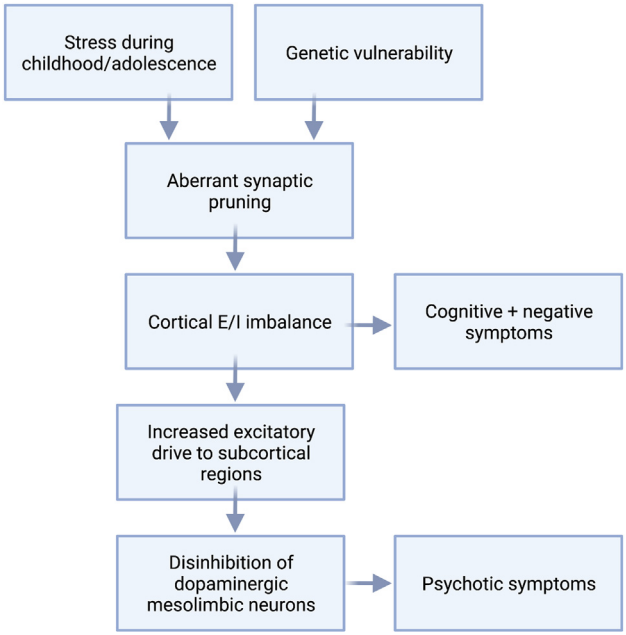

Figure 5. Integrative hypothesis showing how E/I imbalance could lead to onset of cognitive symptoms (e.g., impairments in working memory, processing speed, and executive function) and negative symptoms (e.g., amotivation and flattening of emotions) of schizophrenia as well as to striatal dopaminergic dysfunction, which underlies psychotic symptoms. E/I, excitation-inhibition.

interneurons (85), SV2A (Syndesi Therapeutics), and GABA and nicotinic systems (Recognify Life Sciences).

Another novel treatment pathway is to address aberrant pruning. Minocycline is an antibiotic that inhibits microglial activation, among other actions (147). A two-hit animal model showed that minocycline during stress exposure (the second hit) inhibited microglial activation and prevented behavioral disturbances (148). A study also showed that minocycline or doxycycline exposure for at least 90 days during adolescence was associated with a lower risk for psychosis (49). In contrast, trials of minocycline as an adjunctive treatment in schizophrenia have been mixed (149,150), suggesting that more specific treatments may be needed.

CONCLUSIONS

Schizophrenia is associated with a genetic predisposition affecting proteins involved in excitatory and inhibitory signaling and with postmortem and in vivo evidence for this. Evidence of lower synaptic density and progressive gray matter changes in the disorder suggest that there is disruption in synaptic formation and elimination, particularly in the frontal cortex, although the timing of this remains to be established. We propose that overpruning of cortical glutamatergic synapses during adolescence may tip vulnerable circuits into E/I imbalance, leading to the onset of cognitive and negative symptoms of schizophrenia beginning in the prodrome. Evidence linking frontal cortical abnormalities to disinhibition of mesolimbic striatal dopamine signaling suggests that this process may underlie the eventual onset of psychotic symptoms. In vivo evidence shows that stress during adolescence results in increased synaptic elimination and E/I imbalance. This may be the mechanism through which environmental risk factors predispose someone to develop schizophrenia. This model ties the neurodevelopmental and dopamine hypotheses of schizophrenia into a single pathoetiological hypothesis and identifies preventive therapies targeting pruning and those correcting frontal E/I imbalance as important avenues for future research.

ACKNOWLEDGMENTS AND DISCLOSURES

We thank Dr. Robert McCutcheon for his critical reading of the manuscript.

ODH is a part-time employee of H Lundbeck A/S. He has received investigator-initiated research funding from and/or participated in advisory/speaker meetings organized by Angellini, Autifony, Biogen, Boehringer Ingelheim, Eli Lilly, Heptares, Global Medical Education, Invicro, Jansenn, Lundbeck, Neurocrine, Otsuka, Sunovion, Recordati, Roche, and Viatris/Mylan. Neither ODH nor his family have holdings/a financial stake in any pharmaceutical company. ODH has a patent for the use of dopaminergic imaging. ES reports no biomedical financial interests or potential conflicts of interest. The views expressed are those of the authors and not necessarily those of H Lundbeck A/s, the NHS/NIHR, or the Department of Health.

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Received Nov 16, 2021; revised May 16, 2022; accepted Jun 4, 2022.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2022.06.017.

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