Integrating the neurodevelopmental and dopamine hypotheses of schizophrenia and the role of cortical excitation-inhibition balance.

Oliver D. Howes, Ekaterina Shatalina

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Title: Integrating the neurodevelopmental and dopamine hypotheses of schizophrenia and the role of cortical excitation-inhibition balance.

Short title: Neurodevelopment, dopamine and E/I in schizophrenia

Authors: Oliver D Howes\textsuperscript{1,2}, Ekaterina Shatalina\textsuperscript{1}

Affiliations: \textsuperscript{1}Psychiatric Imaging Group, MRC London Institute of Medical Sciences, Hammersmith Hospital, Imperial College London, London, UK, \textsuperscript{2}Department of Psychosis, Institute of Psychiatry, Psychology and Neuroscience, Kings College London, UK

Corresponding Author:

Prof Oliver Howes

Department of Psychosis Studies, Institute of Psychiatry, Psychology & Neuroscience, King’s College London, 16 De Crespigny Park, London SE5 8AB, UK

Email: oliver.howes@kcl.ac.uk

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Abstract

The neurodevelopmental and dopamine hypotheses are leading theories of the pathoetiology of schizophrenia but were developed in isolation. However, since they were originally proposed, there have been considerable advances in understanding of the normal neurodevelopmental refinement of synapses, and cortical excitatory-inhibitory (E/I) balance, as well as preclinical findings on the inter-relationship between cortical and sub-cortical 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 and glutamatergic signalling, as well as neurodevelopmental processes. Moreover, in vivo studies on the effects of stress, particularly during later development, show 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.

Taken together, this integrated neurodevelopmental and dopamine hypothesis suggests overpruning of synapses, potentially including glutamatergic inputs onto frontal cortical interneurons, disrupts excitation-inhibition balance to underlie 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.
Introduction

Schizophrenia is a common and disabling mental illness that is associated with psychotic, negative and cognitive symptoms, such as impairments in executive function and working memory (1). Two key hypotheses for schizophrenia pathoetiology are the dopamine (2), and neurodevelopmental hypotheses (3, 4). The latter has recently been reframed as the 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 excitatory-inhibitory balance in schizophrenia, studies modelling synaptic pruning mechanisms, genome-wide association studies and novel imaging techniques localising synaptic markers, show how these hypotheses may be integrated with previous work on excitation-inhibition balance (6-8). Here, we first review normal synaptic development, and evidence for neurodevelopmental abnormalities in schizophrenia, before considering the evidence for excitation-inhibition imbalance in schizophrenia, and then propose a new integrative hypothesis that ties together dopaminergic dysfunction with the neuro(socio)developmental hypothesis of schizophrenia.

Synaptic dynamics during neurodevelopment

In rodents and non-human primates, studies show 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 timepoints for different brain regions, in a caudo-rostral manner, with the somatosensory and visual regions amongst the first to reach synaptic stability and the frontal cortex developing last (14).

Human post-mortem 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 developmental 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 also been directly aligned with rodent V1, with synaptic protein expression data suggesting 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 grey matter volumes increase rapidly during childhood followed by reductions during puberty and early adolescence (19, 20). Importantly, different brain regions differ in when grey matter markers reach their peak, start to fall and then stabilize, with higher-order association areas such as the dorsolateral prefrontal cortex maturing later than sensory areas (19, 21, 22), showing the same pattern of tempororo-regional structural changes seen in preclinical research (20, 23) and human post-mortem studies of synaptic measures (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 compared to controls (25). Specifically, patients have been reported to have reduced folding of the anterior cingulate cortex (ACC) (25, 26), and other alterations suggesting impaired gyral formation in 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 grey matter volumes, in particular, in the frontal cortex, relative to controls (29, 30). The progressive loss of grey matter exceeding normal age-related changes in schizophrenia indicates a neurodegenerative process, albeit one that does not result in neuronal death (31, 32). Grey matter reduction in the absence of neuronal loss is consistent the loss of synapses, but it is important to recognise that other changes could contribute to grey matter changes in schizophrenia, such as reduced neuronal processes and branching (33). Further analyses found greater grey matter loss was directly associated with greater duration of illness (34, 35), suggesting there is at least a component of grey matter changes that occurs once the illness has developed. There have now been a number of longitudinal studies testing this further by measuring changes in grey matter volumes over the course of illness from the first episode of psychosis (FEP) (36). These have found patients show accelerated reductions in grey matter volumes in comparison to both their healthy siblings (37) and to matched healthy controls (37-39). One issue with these findings is the potential role of antipsychotic treatment on grey matter changes. However, follow up of patients that start treatment suggests that while medication may have some contribution to grey matter reductions, an appreciable component of grey matter change is not explained by treatment (40, 41).

Taken together, the gyrification and grey matter findings thus 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 do 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

Post-mortem 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 mRNA levels in post-mortem samples from patients with schizophrenia relative to healthy controls, specifically in the hippocampus, frontal and cingulate cortices (42). Another recent meta-analysis looking at post-synaptic density markers also identified reductions in synaptic markers in frontal regions in schizophrenia relative to controls (43).

Further evidence comes from in vivo work, using \[^{11}\text{C}]\text{UCB-J PET imaging, which measures the}\) \[^{11}\text{C}]\text{UCB-J PET imaging, which measures the}\) distribution of synaptic vesicle protein 2A (SV2A). SV2A is ubiquitously expressed in synaptic vesicles and thus differences in protein levels can reflect differences in synaptic density (44, 45). To date, two studies have been published comparing chronic patients with schizophrenia with controls. Both showed significantly lower SV2A density in the patient groups in the frontal and anterior cingulate cortices (46, 47). These and the post-mortem studies, thus, provide evidence for a failure to form and/or loss of synapses in schizophrenia in frontal cortex, and potentially other brain regions. Moreover, further analyses have shown there is altered relationship between SV2A and glutamate levels in patients with schizophrenia (48). Work 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 iPSCs were cultured from patients with schizophrenia compared to those cultured from matched controls (49-51) (for further details see (52)). However, it is important to note that, whilst 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 post-mortem and in vivo lines of evidence indicate altered synaptic elimination in the frontal cortex may affect excitatory (glutamatergic) synapses. However, as GABA was not measured in the in vivo study, further work is required to determine if inhibitory terminals are also affected, and, if so, how this compares to glutamatergic effects in vivo. In view of this, we now consider excitatory-inhibitory balance and how it may be altered in schizophrenia.

Excitatory-inhibitory balance

Excitatory-inhibitory (E/I) balance refers to the relative contribution of excitatory and inhibitory synaptic inputs to brain signalling (53). The integration of these inputs is required for effective
information processing carried out by the brain and occurs at the level of individual neurones, localised 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 caudo-rostral manner, following a similar trajectory to synaptic markers during brain development described above, with the frontal cortex maturing last (55, 56). During this time key mechanisms are upregulated to prevent runaway signalling, whilst achieving a high cortical signal to noise ratio (53). These 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., (2011) have shown that synaptic elimination is facilitated by microglia (57). 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 behaviour (60).

Genetic risk and excitatory and inhibitory neurotransmission in schizophrenia

Genome-wide association studies (GWAS) have shown that schizophrenia is a polygenic disorder, with multiple low-penetrance variants contributing to the genetic risk for schizophrenia (1). One of the most significant genetic associations with schizophrenia implicates genes of the major histocompatibility locus (MHC) locus encoding adaptive immune system components. This in part arises from the presence of many structurally diverse alleles of a complement protein (C4A) that tags synapses for elimination by microglia (61). As well as this, several other genes with roles in microglia-mediated pruning have been identified in GWAS (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 summarised in table 1 and with further detail in supplementary table 1.

Key loci associated with schizophrenia risk linked to excitatory neurotransmission include components of the NMDA receptor (Subunit 2A), 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 (SRR), which catalyses synthesis of the glutamate co-agonist, D-serine, as well as genes encoding components of post-synaptic protein scaffold
of excitatory synapses, including post-synaptic density protein 93 (PSD-93) and SYNGAP1, which is thought to be involved in NMDA-R-dependent control of AMPA-R 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 ANK3 (Ankyrin-G), which promotes stability of somatodendritic GABA-ergic synapses (62, 65, 66). FURIN, a protein involved in GABA-ergic transmission also influences expression of GABA-A receptor components and has been implicated in schizophrenia GWAS 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, summarised in figure 2, indicate that genetic risk for schizophrenia affects proteins involved in both excitatory and inhibitory signalling, which together could predispose an individual to E/I imbalance, although the direction of the imbalance cannot be inferred based on genetics data alone. This imbalance could occur through effects on 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, as 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.

In vivo evidence for altered E/I balance in schizophrenia

Electroencephalography (EEG) and magnetoencephalography (MEG) 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 signalling (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 which are located on glutamatergic afferents and 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 (SICI), long interval intracortical inhibition (LICI) and intracortical facilitation (ICF), respectively (78) (additional details in supplement). These responses have been shown to be reduced in patients with schizophrenia in comparison to controls (79). Another measures, the mismatch negativity (MMN) response, is dependent
on intact NMDA receptor signalling (80, 81). Meta-analysis shows MMN is lower in patients with schizophrenia compared to healthy controls with a large effect size (82), with a recent study showing reduced MMN amplitude was associated with reduced glutamate levels in this patient group, measured with magnetic resonance spectroscopy (83). This is consistent with findings of lower NMDA receptor levels in schizophrenia (84). Notwithstanding this, post-mortem studies show lower levels of GABAergic markers in cortical brain regions (85). While the above 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 MEG/EEG signal. Computational modelling of EEG data from schizophrenia patients suggests 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 with impaired executive function (87). Both altered gamma oscillatory activity (71, 88) and dorsolateral prefrontal cortical SICI responses are correlated with cognitive function in schizophrenia (89). Recent work has also shown working memory deficits following administration of ketamine, a NMDA-R antagonist, to non-human primates. These resembled deficits seen in schizophrenia and were accompanied by decreased inhibitory interneuron and increased excitatory activity in lateral prefrontal cortex (90). These findings, thus, indicate that E/I imbalance could underlie cognitive impairments in schizophrenia. We consider the key question of how these cortical impairments may also lead to psychotic symptoms in the following sections.

Dopamine abnormalities in schizophrenia

Multiples lines of evidence from genetic, post-mortem, 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 D2/3 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 (100, 104, 105), although not in all studies; 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 prefrontal cortex (mPFC) 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 frontal cortex (112). Progressive spine loss in this model led to increased striatal dopamine levels comparable to those from optogenetic simulation of frontal cortical-VTA/SNc circuitry (112). The study also showed that both frontal optogenetic stimulation and progressive cortical synaptic loss lead to hyperlocomotion as well as increased striatal dopamine (112).

NMDA-R antagonists such as ketamine cause negative, cognitive and positive symptoms in healthy volunteers and worsen symptoms in patients with schizophrenia (113). Mice treated with sub-chronic 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 DA neuron function (114). Sub-chronic 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 controls 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 grey matter volume in patients with schizophrenia (117). Furthermore, N-acetylaspartate (NAA) levels in the dorsolateral PFC were associated with greater amphetamine-induced release of striatal dopamine in patients with schizophrenia, but not in healthy controls (118). As lower NAA levels are associated with neuronal dysfunction (119), this suggests 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 controls (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 excitatory-inhibitory balance and synaptic density

Rodent studies show 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 prefrontal cortex (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 remodelling suggest 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 also 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 microglia may mediate aberrant synaptic pruning that lead to E/I imbalance.

Interestingly, numerous rodent studies show effects on E/I balance and enhanced microglial pruning and resultant synaptic loss are more marked in males (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 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, 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 signalling. 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 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. This is anticipated to lead to increased noise in cortical circuits, impairing cortical function and lead to the cognitive and negative symptoms of the disorder. We propose this also disinhibits excitatory projections that regulate mesostriatal dopamine neurons, resulting in dopamine dysregulation and psychotic symptoms through disrupting prediction error signalling (for 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 fit with the time course for the development of symptoms, which typically begin with cognitive impairments, 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 if 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. Key questions are, thus, the precise localization of E/I imbalance within cortical circuitry, the direction of the shift in E/I at different developmental timepoints, 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, while there is some supporting evidence from in vivo and in vitro studies (140, 141), further work is required to replicate these findings and investigate if 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 if 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 signalling contribute to adaptive compensatory change or towards pathology. Additionally, 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, whilst 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 if 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 modelling circuit formation may be useful to better model this developmental stage in schizophrenia. It should also be recognised that synaptic plasticity, and not just absolute synaptic density, is important to cognitive development (144).

One final issue is that, whilst, as we have highlighted, there are data showing 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 leads to striatal hyperactivity via PFC overactivity and if overpruning in schizophrenia may continue into adulthood.

Lastly, stress is a risk factor for many other psychiatric disorders. Why then does this 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 recognised that, whilst 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 ideas that there are neurobiological sub-types 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 pro-cognitive compounds that could do this in development, such as modulators of inhibitory interneurons (85), synaptic vesicle proteins (Syndesi Therapeutics), or GABA and nicotinic systems (Recognify Life Sciences).

Another novel treatment pathway is to address aberrant pruning. Minocycline is an antibiotic which inhibits microglial activation, amongst other actions (147). A two-hit animal model showed that minocycline during stress-exposure (the second hit) inhibited microglial activation and prevented
behavioural disturbances (148). A cohort 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 more specific treatments may be needed.

**Conclusions**

Schizophrenia is associated with a genetic predisposition affecting proteins involved in excitatory and inhibitory signalling and with post-mortem and in vivo evidence for this. Evidence of lower synaptic density and as progressive grey matter changes in the disorder, suggest 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 signalling suggests this process may then underlie the eventual onset of psychotic symptoms. In vivo evidence shows 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 developing schizophrenia. Together, this model ties the neurodevelopmental and dopamine hypotheses of schizophrenia into a single pathoaetiological hypothesis and identifies preventative therapies targeting pruning or those correcting frontal E/I imbalance as important future avenues for research.
Tables and figures:

Table 1: Loci associated with schizophrenia identified by genome wide association studies (GWAS) that have a functional role in excitatory and inhibitory signalling or synaptic pruning.

<table>
<thead>
<tr>
<th>Gene (protein)</th>
<th>Functional role</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genes for proteins involved in excitatory neurotransmission</strong></td>
<td></td>
</tr>
<tr>
<td>ADAM10</td>
<td>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</td>
</tr>
<tr>
<td>AKT3</td>
<td>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.</td>
</tr>
<tr>
<td>CACNA1</td>
<td>Pore-forming, alpha-1C subunit of the voltage-gated calcium channel that gives rise to L-type calcium currents.</td>
</tr>
<tr>
<td>CACNA1D</td>
<td>L-type voltage-gated calcium channel alpha 1D subunit</td>
</tr>
<tr>
<td>CACNA1</td>
<td>Calcium Voltage-Gated Channel Subunit Alpha1 1, T-type calcium channel subunit, involved in neuronal calcium signalling</td>
</tr>
<tr>
<td>CACNB2</td>
<td>Voltage-dependent L-type calcium channel subunit beta-2, Component of a calcium channel complex, involved in neuronal calcium signalling</td>
</tr>
<tr>
<td>DLG2</td>
<td>Discs large MAGUK scaffold protein 2 (DLG2) is part of the postsynaptic protein scaffold of excitatory synapses, and involved in NMDA signalling</td>
</tr>
<tr>
<td>FLOT1</td>
<td>Flotillin-1 (FLOT1) enhances the formation of glutamatergic synapses but not GABAergic synapses (2). Flot1 has been shown to be essential for amphetamine-induced reverse transport of DA in neurons but not for DA uptake (1)</td>
</tr>
<tr>
<td>GRIA1</td>
<td>Glutamate Ionotropic Receptor AMPA Type Subunit 1</td>
</tr>
<tr>
<td>GRIN2A</td>
<td>Glutamate Ionotropic Receptor NMDA Type Subunit 2A</td>
</tr>
<tr>
<td>GRM3</td>
<td>Glutamate Metabotropic Receptor 3</td>
</tr>
<tr>
<td>HCN1</td>
<td>The hyperpolarization-activated cyclic nucleotide-gated (HCN1) channels modulate the rate of glutamate release by changing rate of exocytosis in synaptic terminals.</td>
</tr>
<tr>
<td>Protein</td>
<td>Description</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>RYR3</td>
<td>Ryanodine receptor type 3 (RyR3) involved in Ca signalling</td>
</tr>
<tr>
<td>SRR</td>
<td>Serine racemase catalyzes the synthesis of D-serine from L-serine. D-serine is a key coagonist with glutamate at NMDA receptors</td>
</tr>
<tr>
<td>SYNGAP1</td>
<td>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.</td>
</tr>
<tr>
<td>ANK3</td>
<td>Ankyrin-G/ankyrin-3 (ANK3) is integral to AMPAR-mediated synaptic transmission and maintenance of spine morphology (1). It promotes stability of somatodendritic GABAergic synapses \textit{in vitro} and \textit{in vivo} through opposing endocytosis of GABA-A receptors (2)</td>
</tr>
<tr>
<td>CLCN3</td>
<td>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.</td>
</tr>
<tr>
<td>FURIN</td>
<td>Furin a protease enzyme is involved in GABA-A-mediated synaptic transmission.</td>
</tr>
<tr>
<td>GABBR1</td>
<td>Gamma-Aminobutyric Acid Type B Receptor Subunit 1</td>
</tr>
<tr>
<td>GABBR2</td>
<td>Gamma-Aminobutyric Acid Type B Receptor Subunit 2</td>
</tr>
<tr>
<td>PLCL1</td>
<td>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.</td>
</tr>
<tr>
<td>SLC32A1</td>
<td>Solute Carrier Family 32 Member 1 is Involved in the uptake of GABA and glycine into the synaptic vesicles.</td>
</tr>
<tr>
<td>ADAM10</td>
<td>ADAM metalloprotease 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.</td>
</tr>
<tr>
<td>CSMD1</td>
<td>Regulator of C4 expression</td>
</tr>
<tr>
<td>C4</td>
<td>Complement component 4, protein expressed on synapses to tag them for elimination by microglia</td>
</tr>
<tr>
<td>Gene</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>PDE4B</td>
<td>Phosphodiesterase 4B is a microglia target to reduce neuroinflammation. Also expressed at the synapse.</td>
</tr>
<tr>
<td>VRK2</td>
<td>Vaccinia-related kinase 2 plays a critical role in microglia-mediated synapse elimination during neurodevelopment.</td>
</tr>
<tr>
<td></td>
<td>Genes for proteins involved in establishing E/I balance during neurodevelopment</td>
</tr>
<tr>
<td>AMBRA1</td>
<td>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.</td>
</tr>
<tr>
<td>CLSTN3</td>
<td>Calsyntenin-3 promotes inhibitory and excitatory synaptic development</td>
</tr>
<tr>
<td>CUL3</td>
<td>Culin-3 is compartmentalized at postsynaptic densities and gates retrograde signalling, it is involved in neural development, neurotransmission, and maintaining excitation-inhibition (E-I) balance and glutamate receptor turnover.</td>
</tr>
<tr>
<td>FOXP1</td>
<td>Forkhead box protein 1 is a transcription factor for genes associated with synaptic function and development.</td>
</tr>
<tr>
<td>GPM6A</td>
<td>Glycoprotein M6A contributes to spine and, likely, synapse formation</td>
</tr>
<tr>
<td>HIP1R</td>
<td>Huntingtin-Interacting Protein 1-Related Protein plays a critical role in dendritic development and excitatory synapse formation in hippocampal neurones</td>
</tr>
<tr>
<td>IGSF9B</td>
<td>Immunoglobulin Superfamily Member 9B is a transmembrane protein which is abundantly expressed in interneurons, where it may regulate inhibitory synapse development</td>
</tr>
<tr>
<td>KALRN</td>
<td>Kalirin7 is involved in the formation of dendritic spines</td>
</tr>
<tr>
<td>LRRRTM4</td>
<td>Leucine Rich Repeat Transmembrane Neuronal 4 is involved in regulating excitatory synapse development</td>
</tr>
<tr>
<td>MEF2C</td>
<td>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.</td>
</tr>
<tr>
<td>NLGN4X</td>
<td>Neuroligin 4 X-Linked is a member of the neuroligin family of proteins, which are involved in the regulation of excitatory synaptic transmission.</td>
</tr>
</tbody>
</table>
1. 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, iPSC findings 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 grey matter volume changes in the prodrome and early phase of illness. Based on these findings, we propose that there is also aberrant synaptic pruning both early and later in neurodevelopment, leading to overpruning of synapses and excitatory/inhibitory imbalance and schizophrenia. Further patient studies are required to determine the course of synaptic loss.

2. Figure 2: Genes encoding inhibitory and excitatory signalling components identified by schizophrenia genome-wide association studies associated with schizophrenia risk. GRM3 - Glutamate Metabotropic Receptor 3, AKT3 - AKT serine/threonine kinase 3, DLG2 - Discs large MAGUK scaffold protein 2, GRI2A - Glutamate Ionotropic Receptor NMDA Type Subunit 2A, GRIA1 - Glutamate Ionotropic Receptor AMPA Type Subunit 1, SYNGAP1 - Synaptic Ras GTPase Activating Protein 1, HCN1 - hyperpolarization-activated cyclic nucleotide-gated channel component, SRR - Serine racemase, CACNB2 - Voltage-dependent L-type calcium channel subunit beta-2, CACNA1 - Calcium Voltage-Gated Channel Subunit Alpha1 I, GABBR - Gamma-Aminobutyric Acid Type B Receptor, ANK3 - Ankyrin-G/ankyrin-3, PLCL1 - Phospholipase C Like 1, CLCN3 - Chloride Voltage-Gated Channel 3, SLC31A1 - Solute Carrier Family 32 Member 1.

3. Figure 3: Aberrant excitatory-inhibitory balance in the frontal cortex of patients with schizophrenia. Lower levels of excitatory synaptic inputs onto inhibitory interneurons (shown in green) result in increased activity of pyramidal neurons (shown in orange, arrows indicate activity).

4. Figure 4: Projections from the frontal cortex to the striatum and midbrain origin of dopamine neurons. Frontal excitatory-inhibitory (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.

5.

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

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Ekaterina Shatalina has reported 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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Stress during childhood/adolescence

Genetic vulnerability

Aberrant synaptic pruning

Cortical E/I imbalance

Cognitive + negative symptoms

Increased excitatory drive to subcortical regions

Disinhibition of dopaminergic mesolimbic neurons

Psychotic symptoms