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Relevance of Interactions Between Dopamine and Glutamate Neurotransmission in Schizophrenia

Silas A. Buck[1,2], **M. Quincy Erickson-Oberg**[1,2], **Ryan W. Logan**[3,4], **Zachary Freyberg**[2,5,*]

[1]Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213, USA

[2]Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15213, USA

[3]Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118, USA

[4]Center for Systems Neuroscience, Boston University, Boston, MA 02118, USA

[5]Department of Cell Biology, University of Pittsburgh, Pittsburgh, PA 15213, USA

Abstract

Dopamine (DA) and glutamate neurotransmission are strongly implicated in schizophrenia pathophysiology. While most studies focus on contributions of neurons that release only DA or glutamate, neither DA nor glutamate models alone recapitulate the full spectrum of schizophrenia pathophysiology. Similarly, therapeutic strategies limited to either system cannot effectively treat all three major symptom domains of schizophrenia: positive, negative, and cognitive symptoms. Increasing evidence suggests extensive interactions between the DA and glutamate system and more effective treatments may therefore require the targeting of both DA and glutamate signaling. This offers the possibility that disrupting DA-glutamate circuitry between these two systems, particularly in the striatum and forebrain, culminate in schizophrenia pathophysiology. Yet, the mechanisms behind these interactions and their contributions to schizophrenia remain unclear. In addition to circuit- or system-level interactions between neurons that solely release either DA or glutamate, here we posit that functional alterations involving a subpopulation of neurons that co-release both DA and glutamate provide a novel point of integration between DA and glutamate systems, offering a key missing link in our understanding of schizophrenia pathophysiology. Better understanding of mechanisms underlying DA/glutamate co-release from these neurons may therefore shed new light on schizophrenia pathophysiology and lead to more effective therapeutics.

Keywords

Dopamine; glutamate; VGLUT2; co-transmission; schizophrenia

[*]**Corresponding Author:** Zachary Freyberg, M.D., Ph.D., University of Pittsburgh, Department of Psychiatry, 3811 O'Hara Street, Biomedical Science Tower, W1640, Pittsburgh, PA 15213, USA, freyberg@pitt.edu.

Author Contributions

SAB and ZF conceived the idea and designed the manuscript. SAB, MQEO, RWL and ZF wrote the manuscript. SAB, MQEO, RWL and ZF created figures and performed editing. All authors approved the final manuscript version.

Conflicts

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Introduction

Schizophrenia is a prevalent serious mental illness, affecting ~1% of the global population[1]. The combination of positive (*e.g.*, delusions, hallucinations, thought disorganization), negative (*e.g.*, social withdrawal, amotivation), and cognitive (*e.g.*, attention deficits, impaired working memory) symptoms causes substantial disabilities[2]. Antipsychotic drugs remain the primary therapeutic option in schizophrenia. Nevertheless, these drugs remain limited in their abilities to effectively improve negative symptoms and cognition, and often produce metabolic disturbances that limit compliance[1, 3–5]. Furthermore, the mechanisms underlying schizophrenia pathophysiology remain unclear. Thus, an improved understanding of the biology of schizophrenia is needed to generate more effective treatments that ameliorate all three major symptom domains.

Despite increasing awareness of the complex polygenic and environmental interrelationships that contribute to schizophrenia pathogenesis, dysfunction in brain dopamine (DA) and glutamate neurotransmission have remained mainstays of our understanding of the disease. We will examine DA and glutamate systems as well as their interactions in the context of schizophrenia. We will additionally focus on a distinct subpopulation of midbrain DA neurons that co-transmit both DA and glutamate in the striatum and forebrain, regions highly relevant to schizophrenia pathogenesis and its treatment.

Dopamine and glutamate models of schizophrenia

Dysregulated modulation of striatal DA neurotransmission is the central tenet of the DA hypothesis of schizophrenia, the predominant theory of schizophrenia for the last several decades[6–9]. This theory suggests that altered striatal DA neurotransmission within the mesolimbic pathway leads to positive symptoms[7]. Early postmortem human brain studies demonstrated increased striatal DA, later confirmed by *in vivo* PET imaging[7, 8, 10]. Similarly, striatal presynaptic DA function is elevated in individuals with prodromal symptoms of schizophrenia, and conversion from the prodrome to first-episode psychosis is associated with increased striatal DA synthesis[11, 12]. Indeed, mouse models that raise striatal DA signaling via psychostimulants or DA D_2 receptor (D2R) overexpression produce behaviors associated with psychosis (*e.g.,* working memory deficits)[13–16]. Conversely, blockade of striatal DA D_2-like receptors improves positive symptoms, further supporting the importance of DA signaling in schizophrenia symptomatology[7, 17].

Glutamate has also been implicated in schizophrenia pathogenesis[18]. Antagonists of N-methyl-D-aspartate (NMDA) glutamate receptors including ketamine and phencyclidine produce positive, negative, and cognitive symptoms clinically; animal models similarly exhibit schizophrenia-related impairments (*e.g.*, sensorimotor gating and social deficits)[13, 19, 20]. Animals with diminished levels of D-serine, an NDMA receptor co-agonist, exhibit decreased hippocampal volume and dendritic spine density; these pathologic changes are similar to those in brains of individuals with schizophrenia (see Box)[21]. Additionally, induced pluripotent stem cell (iPSC) studies employing neurons derived from individuals with schizophrenia show dysfunctional glutamate receptor signaling, diminished glutamate release, delayed maturation of glutamate neurons, and reduced synaptic connectivity[22–24].

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Postmortem brain studies of individuals with schizophrenia also show that expression of excitatory amino transporter 2 (EAAT2), a transporter required for glutamate uptake, is reduced in temporal and frontal regions versus unaffected comparison subjects[25]. This is accompanied with diminished glutaminase expression in dorsolateral prefrontal cortex (DLPFC) and thalamus in schizophrenia[25]. Overall, these data point to the relevance of both DA and glutamate systems in schizophrenia.

Limitations of DA and glutamate models of schizophrenia

Many questions remain concerning respective contributions of DA and glutamate models to schizophrenia pathophysiology. For the DA model, while antipsychotic drug blockade of D_2-like receptor signaling is linked to positive symptom reduction, these medications do not effectively address either the negative or cognitive symptoms of the illness[38, 39]. Likewise, despite the glutamate model's reproduction of some negative and cognitive symptoms in various animal systems, it is limited in effectively reproducing the disturbances in DA neurotransmission indicative of schizophrenia or in explaining the clinical efficacy of DAergic antipsychotic drugs[13]. This suggests that, individually, these animal models are unlikely to fully recapitulate the primary pathology related to schizophrenia resulting in both DAergic and glutamatergic dysfunction. Additionally, evidence for the efficacy of glutamatergic drugs in schizophrenia treatment has been mixed[38]. Though some clinical studies suggest that NMDA receptor modulators including D-serine and sarcosine improve positive and negative symptoms, other work shows no benefit[38, 40, 41]. Similarly, there were initially promising results in a small randomized, double blind, placebo-controlled clinical trial comparing efficacy of LY2140023, a metabotropic glutamate 2/3 receptor (mGluR2/3) agonist[42]. However, a subsequent larger multicenter, randomized, double-blind, parallel, placebo-controlled trial was inconclusive since neither LY2140023 nor the olanzapine positive control differed significantly versus placebo[42, 43]. These data suggest that neither DA nor glutamate models on their own sufficiently explain the full spectrum of schizophrenia pathogenesis and that our existing pharmacological and animal models provide only partial answers. Instead, we posit that pathogenic alterations that modify the interactions between DA and glutamate systems alter striatal signaling, ultimately culminating in psychosis as well as negative and cognitive symptoms.

Interactions between DA and glutamate systems in schizophrenia

There is increasing evidence of extensive bidirectional communication between DA and glutamate systems, particularly in the PFC, midbrain, and striatum of humans, nonhuman primates, and rodents[44–48]. Each system impacts its counterpart and disturbances in these interactions have been implicated in schizophrenia pathology[9, 49]. Furthermore, DA-glutamate system interactions are part of a larger framework where additional neurotransmitter systems intersect. For example, serotonin signaling in PFC and striatum modulates both DAergic and glutamatergic systems and interactions between these systems mediates therapeutic efficacy of atypical antipsychotic drugs[50–52]. Similarly, the interplay of glutamatergic and cholinergic systems in cortex and hippocampus is important for working memory and linked to the cognitive deficits of schizophrenia both clinically and in rodent schizophrenia models[53–56].

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In DA/glutamate system interactions, midbrain DA neuron activity is modulated by glutamatergic inputs from the frontal cortex[57–59]. In this circuit: 1) Cortical pyramidal glutamatergic projections stimulate inhibitory GABA interneurons; 2) Release of GABA from the interneurons lowers the firing rate of cortical glutamate neurons that project onto midbrain DA neurons; 3) Modulating glutamate tone from these midbrain projections provides homeostatic control over DAergic firing in the mesolimbic pathway[57] (Figure 1a). Disruptions in DA-glutamate communications may drive schizophrenia pathology, leading to abnormally elevated striatal DA[13, 38, 57]. This forms the foundation of the 'NMDA receptor hypofunction hypothesis' which asserts that hypofunctional NMDA receptor signaling in cortical GABAergic interneurons decrease GABAergic inhibitory tone to increase firing by midbrain-projecting secondary glutamatergic neurons (Figure 1b). This increases midbrain DA neuron stimulation to raise striatal DA associated with positive symptoms[57, 59].

The DA system plays a similarly important role in modulating glutamatergic circuitry. Ascending DAergic projections to the PFC impact the glutamatergic circuitry that coordinate working memory and executive function[60, 61]. This DA signaling occurs via stimulatory D_1 (D1R) as well as inhibitory D2R and D_3 (D3R) receptors, which are commonly co-expressed in glutamatergic PFC neurons[62]. This combination of stimulatory and inhibitory DA receptors exerts opposing effects on extracellular levels of D-serine to fine-tune the local glutamatergic neurotransmission[60]. Such DA-mediated fine-tuning in the PFC has important functional implications where DA levels and cognitive performance follow an inverted U-shaped function: either too little or too much DA receptor activation impairs cognition[63, 64]. Therefore, we posit that changes in the coordination between DAergic signals may precipitate disturbances in the downstream glutamatergic circuitry to fuel schizophrenia pathology (Figure 1c). Such a mechanism provides a key link between DA- and D-serine-mediated NMDA receptor signaling and the cognitive symptoms in schizophrenia. Moreover, since the antipsychotic drug clozapine raises D-serine release in the frontal cortex and modifies local PFC D_2-like receptor signaling, this may reestablish the balance of DAergic fine-tuning, providing targets for development of more drugs that can better target the cognitive sequelae of schizophrenia[60, 65–67] (Figure 1c).

Abnormal sensorimotor gating and accompanying hippocampal hyperactivity, robust schizophrenia endophenotypes[68], provide additional evidence of interactions between DA and glutamate systems. Data from rodent models shows a mechanistic relationship between hippocampal hyperactivity involving glutamatergic circuits and dysregulated DA release from midbrain DA neurons projecting to the striatum. Raising ventral hippocampus or ventral subiculum output leads to increased DA signaling, resulting disruptions in sensorimotor gating including reduced pre-pulse inhibition (PPI)[68–70]. Similarly, in a 22q11 deletion syndrome mouse model of schizophrenia, elevated DA D_2 receptor-mediated DA signaling in thalamic neurons disrupts glutamatergic neurotransmission from thalamocortical neurons that project to the auditory cortex[71]. Conversely, acute treatment with NMDA receptor antagonist ketamine raises striatal and cortical DA release in rodents, consistent with the NMDA receptor hypofunction hypothesis[72]. In humans, magnetic resonance spectroscopy (^{1}H-MRS) studies showed that amphetamines which raise striatal DA also increase glutamate levels in the dorsal anterior cingulate cortex (dACC) in a sex-specific manner in healthy subjects[73]. While amphetamine increases dACC glutamate similarly in

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men and women, methamphetamine differentially augments glutamate levels in women versus men[73]. This is consistent with increased vulnerability to methamphetamine and methamphetamine-induced euphoria in women[74, 75]. Overall, close integration between DA and glutamate systems may also more accurately explain why alterations primarily to DA or glutamate signaling alone are insufficient to reproduce the deficits in all three schizophrenia symptom domains.

Overview of DA/glutamate co-transmission

To date, most studies examining DA and glutamate systems have focused on neurons that primarily release either DA or glutamate. We propose that co-transmission of glutamate alongside DA from a population of the *same* midbrain DA neurons may represent an important but previously underappreciated aspect of schizophrenia pathophysiology. Historically, Dale's principle maintained that a single neurotransmitter is released from all synaptic terminals of a neuron – a concept accepted as dogma for decades[76]. It has since been demonstrated that more than one neurotransmitter can be released from a single neuron[77, 78]. This paradigm is embodied by co-transmission of both DA and glutamate from a distinct subpopulation of midbrain DA neurons that also express machinery of glutamatergic neurotransmission including the vesicular glutamate transporter 2 (VGLUT2). VGLUT2-expressing DA/glutamate neurons are mainly found in the medial ventral tegmental area (VTA) and project to the nucleus accumbens (NAc) medial shell; an additional population of DA/glutamate cells localizes to the lateral substantia nigra *pars compacta* (SNc) and projects to the tail of the striatum[79–85].

VGLUT2 expression is dynamic across DA neuron development[86]. In mouse mesencephalon and diencephalon development, VGLUT2 is detected by embryonic day 9.5, which precedes expression of DA neuron markers NURR1 and tyrosine hydroxylase (TH)[87], suggesting that VGLUT2 is an early marker of DA neuron development. Importantly, mouse studies show that the majority of SNc DA neurons repress VGLUT2 expression by postnatal day 14, raising the possibility that DA neuron VGLUT2 expression is part of a regulated neurodevelopmental program linking DA and glutamate systems[87, 88].

Compared to rodents, considerably less is known about the developmental course of VGLUT2 in human brain. Availability of human brain gene expression repositories enabled us to examine regional VGLUT2 expression across human brain development and the lifespan. Per the Brainspan Atlas of the Developing Human Brain, VGLUT2's expression levels in striatum are high early in development *in utero* and prior to parturition, followed by a significant drop for the rest of life. This pattern is consistent with the Human Brain Transcriptome atlas where VGLUT2 expression is high in fetal brain across most brain regions (*e.g.*, neocortex, hippocampus, amygdala, striatum, cerebellar cortex), followed by a rapid decrease in expression shortly following birth. A limitation of these analyses is the absence of data for VGLUT2 expression specifically in DA neurons. Future studies will focus on mapping VGLUT2 expression in human midbrain $TH^+/VGLUT2^+$ DA neurons.

Dynamic changes in DA neuron VGLUT2 expression also occur in the adult human brain as a neuroprotective response to insults. For example, in subjects with Parkinson's disease,

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VGLUT2 expression is upregulated in surviving midbrain DA neurons[89]. Similar findings in rodent and *Drosophila* models show DA neurons that upregulate VGLUT are more resilient[84, 85, 88, 90]. Overall, these models show that temporal control over DA neuron VGLUT2 expression is highly regulated and conserved[87, 88, 91–95]. Such control is likely relevant to the overlapping circuitry between DA and glutamate systems since VGLUT2 expression increases DA axon arborization[92]. Subsets of VTA DA neurons that retain VGLUT2 expression through adulthood employ VGLUT2 to maintain the complex axonal branching necessary for synaptic communication. Conversely, disruption of VGLUT2's regulatory program in DA neurons either in development or postnatally may lead to dysfunction in striatal circuits that rely on DA and glutamate neurotransmission. These data raise the question: what roles might co-transmission of DA and glutamate play in the context of schizophrenia?

Relevance of VGLUT2 and DA/glutamate co-transmission in schizophrenia

Important clues concerning the relevance of DA/glutamate co-release to schizophrenia have emerged in recent years based on work in *Drosophila* and mouse models. These studies suggest that VGLUT2-driven regional alterations in patterns of DA neuron activity and the associated DA release from striatal terminals may drive schizophrenia pathogenesis[96]. In *Drosophila*, we showed *Drosophila* VGLUT (dVGLUT) modulates activity-dependent vesicular DA loading and release, allowing neurons to tune presynaptic DA release to changes in cell firing[97]. These VGLUT-mediated effects are also evident in mouse midbrain DA neurons[97], reflecting VGLUT's importance in modulating DA release. The mechanism for this conserved phenomenon is based on VGLUT's ability to hyperacidify DA synaptic vesicles (SVs) in response to increased activity via vesicular synergy[96, 98]. Since the vesicular pH gradient is the primary driving force for vesicular DA loading, VGLUT2-mediated vesicle hyperacidification elevates DA loading and increases release during neuronal depolarization[97, 99, 100]. Disrupting DA neuron VGLUT expression via conditional knockout (cKO) in mice or RNAi knockdown in flies significantly alters vesicular DA loading and psychostimulant-induced behaviors, emphasizing VGLUT's relevance to DAergic neurotransmission[81, 97, 101]. Moreover, in *Drosophila*, dVGLUT functions in DA neurons as a rheostat of DAergic neurotransmission. When synaptic DA levels drop, DA neurons upregulate dVGLUT expression as a homeostatic compensatory response to maintain relatively stable DA neurotransmission[85]. While the *Drosophila* model has provided many insights, caution must be taken in directly extrapolating observations in the fly to human psychiatric disorders. The fly model lacks homologs of some vertebrate-specific pathogenic factors evident in schizophrenia such as *DISC1*[102]. It is also more difficult to model complex behaviors including those related to cognition and fly brain architecture differs from mammalian models. Finally, monoamine pharmacology differs between *Drosophila* and vertebrates, with flies substituting the trace amines octopamine and tyramine for norepinephrine. Together, these factors underscore potential drawbacks in modeling human disease in *Drosophila* and emphasize the need to validate findings from the fly with mammalian models.

Additional questions remain concerning VGLUT2's localization in DA neurons. Initial studies in rat ventral striatum assessing SV colocalization of VGLUT2 and

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vesicular monoamine transporter 2 (VMAT2), which concentrates DA into SVs, demonstrated that most VMAT2$^+$ immunoreactive vesicles possessed VGLUT2 in reciprocal immunoprecipitation experiments. These data suggested that VGLUT2 colocalizes with a discrete fraction of VMAT2$^+$ vesicles, consistent with evidence that glutamate stimulates VMAT2-mediated uptake in rat SVs[103]. Subsequent studies in *Drosophila* similarly showed co-localization of *Drosophila* VMAT and dVGLUT at specific sites in adult fly brain[97]. However, other work in mice, rats, and hamsters found negligible co-localization between VGLUT2 and VMAT2 in midbrain DA neuron projections to striatum. Instead, these studies demonstrated that most glutamatergic and DAergic SVs segregate to separate release sites[84, 104–106]. Insights into reconciling these discrepancies come from new combined super-resolution imaging and immunolabeling methods that accurately resolve colocalization of vesicular transporters on single SVs[107]. These methods showed that VGLUT2$^+$/VMAT2$^+$ SVs comprise 3–4% of the total VMAT2$^+$ SV pool[107]. Since these results used SV fractions from total rat brain homogenates, we posit that some of the transporter colocalization differences between studies may be related to species- and/or brain region-specificity where some species/brain regions are enriched in VGLUT2$^+$/VMAT2$^+$ SVs whereas other regions are sparser. Emergence of more sensitive methodologies enables future comprehensive mapping of this SV subpopulation in the context of schizophrenia, particularly in regions such as PFC where VGLUT2/VMAT2 vesicular colocalization has yet to be identified.

We propose that the co-release of DA and glutamate from the same neurons enables finely tuned integration of DAergic and glutamatergic signaling with exquisite temporal and spatial specificity. We also speculate that elevations in DA neuron VGLUT2 expression further boost striatal DA levels during periods of neuronal activity to contribute to positive symptoms (Figure 2). This agrees with work showing that VGLUT2 levels are elevated in the NAc of individuals with schizophrenia[108–110]. Furthermore, analyses examining single nucleotide polymorphisms (SNPs) within the VGLUT2 promoter and exons of VGLUT2 from genomic data of schizophrenia patients revealed 9 rare genetic variants in the patient group with none in controls[111]. This suggests that some of these SNPs may increase the genetic burden that contributes to schizophrenia pathogenesis. Additionally, in human postmortem brain studies, VGLUT2 transcript expression is elevated in the dorsal thalamus and inferior temporal gyrus in schizophrenia[112, 113]. These elevations in VGLUT2 expression are region-specific as the anterior cingulate cortex, dorsolateral prefrontal cortex, and hippocampus do not exhibit these expression differences versus controls[114, 115]. However, since these studies relied on brain homogenates to detect changes in VGLUT2 expression, the identities of the VGLUT2-expressing projections into these regions remains unclear. Future work will require *in situ* mapping of the pre- and postsynaptic circuitry within the intact respective brain regions.

In addition to DA, striatal glutamate co-released by projections of VGLUT2$^+$ VTA DA/glutamate neurons is relevant both physiologically and in the context of schizophrenia. While glutamatergic inputs to the VTA are well-studied, less is known about roles of glutamatergic inputs to the striatum, particularly in terms of their regulation of DA release and/or medium spiny neuron activation. Work in rodents and guinea pigs shows glutamate released from these inputs inhibits striatal DA release via indirect effects on

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nearby striatal medium spiny neurons (MSNs)[116, 117]. Glutamate released by the striatal glutamatergic inputs causes the following sequence of events: 1) AMPA receptor-mediated MSN activation which 2) increases mitochondrial production of membrane permeable H_2O_2, which 3) rapidly diffuses into adjacent DAergic projections where it inhibits DA release[116–118]. Glutamate released by striatal glutamatergic projections also drives burst firing of striatal cholinergic interneurons (ChI) via AMPA/kainate and NMDA receptor signaling, especially in the NAc medial shell. In mice, this glutamate-driven firing is important for behavioral plasticity that is pathologically impacted by drugs of abuse such as amphetamines[119]. Moreover, VGLUT2 expressed by VTA DA/glutamate neurons mediates this process since VGLUT2 cKO in DA/glutamate neurons attenuates subsequent ChI burst firing[119]. Similarly, glutamate released from VTA neurons that project to the NAc medial shell is important for positive reinforcement and in predicting responses to reward and reward-mediated cues[120–122]. Notably, even when DA co-release is blocked, the glutamate released from striatal projections of VTA DA/glutamate neurons promotes reinforcement[122]. This demonstrates a role for glutamate independent of concurrent DA co-release and further implicates glutamate as having its own novel roles in reward-seeking behaviors. In schizophrenia, though abnormalities in reward processing are classically associated with dysfunctional DAergic activity by VTA projections to the NAc and elevated DA levels, glutamate is increasingly shown to be a key modulator of these processes[123, 124]. Therefore, we speculate that alterations in glutamate release from VTA DA/glutamate neuronal projections to the NAc may contribute to mechanisms mediating reward-related abnormalities in schizophrenia. Given the distinct functional properties of DA and glutamate detailed above, this also raises the question of whether DA/glutamate neurons behave more like purely DAergic neurons versus glutamatergic neurons or have an entirely distinct identity which further work will need to disentangle.

In addition to their striatal projections, midbrain DA/glutamate neurons also project to forebrain regions, including the PFC[86, 125]. In tracing studies, cell bodies of DA/glutamate neurons are situated in specific VTA subdivisions, specifically the rostral VTA, the rostral linear nucleus of the raphe and the parabrachial pigmented nucleus[126, 127]. These cells' projections elicit excitatory postsynaptic potentials in PFC GABAergic interneurons and cingulate cortical pyramidal neurons[86, 128–130]. This raises that possibility that altered DA/glutamate co-transmission in the forebrain leads to the dysfunctional interactions between DA and glutamate signaling that contributes to cognitive symptoms associated with schizophrenia.

Impairments in working memory are established cognitive symptoms in schizophrenia[131, 132]. Recent work in mouse models implicated DA/glutamate co-transmission in working memory deficits, showing that VGLUT2 cKO in DA neurons impairs spatial working memory and disrupts oscillatory activity in the hippocampal CA3 region[133]. Since loss of oscillatory synchrony between hippocampal and prefrontal regions is implicated in schizophrenia-related working memory deficits[134], this offers a new pathological mechanism linking DA neuron VGLUT2-mediated deficits to altered coordination between hippocampal-prefrontal activity. Another key cognitive symptom is disruption in latent inhibition, the ability to discriminate cue saliency. Latent inhibition models attentional abnormalities in schizophrenia and in individuals at high risk for

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psychosis[135–137]. In DA/glutamate neurons, reducing expression of glutaminase, the enzyme responsible for glutamate biosynthesis, causes potentiation of latent inhibition and leads to a schizophrenia resilience phenotype in mice[138]. This suggests a role for DA neuron glutamate in hippocampus-mediated spatial memory, and raises the possibility that DA neuron glutamate biosynthesis may be impaired in schizophrenia[139–144]. Finally, while the relevance of DA/glutamate neuron projections to the PFC on PPI have yet to be directly investigated in human subjects, DA and glutamate systems in the PFC are known to play a major role in mediating deficits in PPI in rodent models of schizophrenia[145–148]. Altogether, reducing glutamatergic co-transmission in DA neurons may provide a novel therapeutic strategy to improve cognitive deficits in schizophrenia. Future work should therefore manipulate DA and glutamate co-transmission in this distinct neuronal subpopulation to target the cognitive symptoms in schizophrenia more effectively. However, to date, all studies of DA/glutamate co-release, VGLUT2 localization on DAergic vesicles and DA/glutamate vesicular synergy have been conducted in the VTA/NAc circuit. Thus, while DA/glutamate neurons are known to project to the PFC and cingulate cortex, the interplay between DA and glutamate transmission of DA/glutamate neurons in these regions is unknown and therefore merits future study.

Conclusions

The original DA hypothesis of schizophrenia asserts that alterations in the balance of DAergic neurotransmission in the mesolimbic and mesocortical pathways are responsible for positive and negative symptoms, respectively. This hypothesis has provided key insights into the circuitry and signaling associated with the positive symptoms of schizophrenia and their treatment with DA D_2-like receptor antagonists. Yet, the DA hypothesis has been insufficient in providing a robust framework for understanding the mechanisms underlying negative or cognitive symptoms which often predominate. Instead, more recent preclinical and clinical studies have given way to a more complete picture – that the DA system extensively interacts with other brain systems including the glutamate system. Thus, disturbances in this DA/glutamate interplay may drive the development of not only positive but also negative and cognitive schizophrenia symptoms. Consistent with this, treatments primarily geared only to targets within the DA or glutamate systems have not been effective in improving all three core domains of schizophrenia. Rather, disruptions in the communications between these systems likely play significant roles in shaping the pathophysiology of schizophrenia.

Nevertheless, many questions remain concerning the roles of the DA and glutamatergic systems in schizophrenia biology. Foremost, it remains unclear whether alterations in striatal DA levels and signaling are the primary drivers of schizophrenia pathophysiology or are a downstream consequence of a dysfunctional glutamate system. Given the heterogeneity of schizophrenia, it is likely that both scenarios are correct. There may be a subset of individuals where primary defects in DA signaling drive the main symptoms of the illness. In other cases, alterations in the DA system may be downstream of other primary causes including within the glutamate system. Another outstanding question concerns identifying the precise circuits linking the DA and glutamate systems and their functional relevance. Lastly, the precise contributions of DA/glutamate co-releasing neurons to schizophrenia remain largely unknown. Therefore, a key aim of this Perspective is to raise the speculative

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hypothesis that DA/glutamate neurons may have a role in schizophrenia and thus motivate the field to actively pursue these important questions. Future work will offer critical new insights on these questions as well as provide new targets that lead to the development of new, more effective therapies for schizophrenia.

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Box: D-Serine

D-Serine is an amino acid synthesized in astrocytes, microglia, and neurons from its enantiomer, L-Serine, by serine racemase[26]. D-Serine is concentrated in human and rodent forebrain regions including PFC, hippocampus, and striatum[27–29]. D-serine's anatomic distribution is closely correlated with expression of its target, the NMDA receptor[28]. D-serine is a potent and selective co-agonist of NMDA receptors[30–32], binding the receptor's glycine site[33]. Given that 1) D-serine is the primary co-agonist of pyramidal neuron NMDA receptors in the prefrontal cortex[29, 34], and 2) genes encoding serine racemase and D-amino acid oxidase (which degrades D-serine) are linked to schizophrenia risk[35, 36], D-serine has become a popular novel candidate for schizophrenia therapeutics[26, 37].

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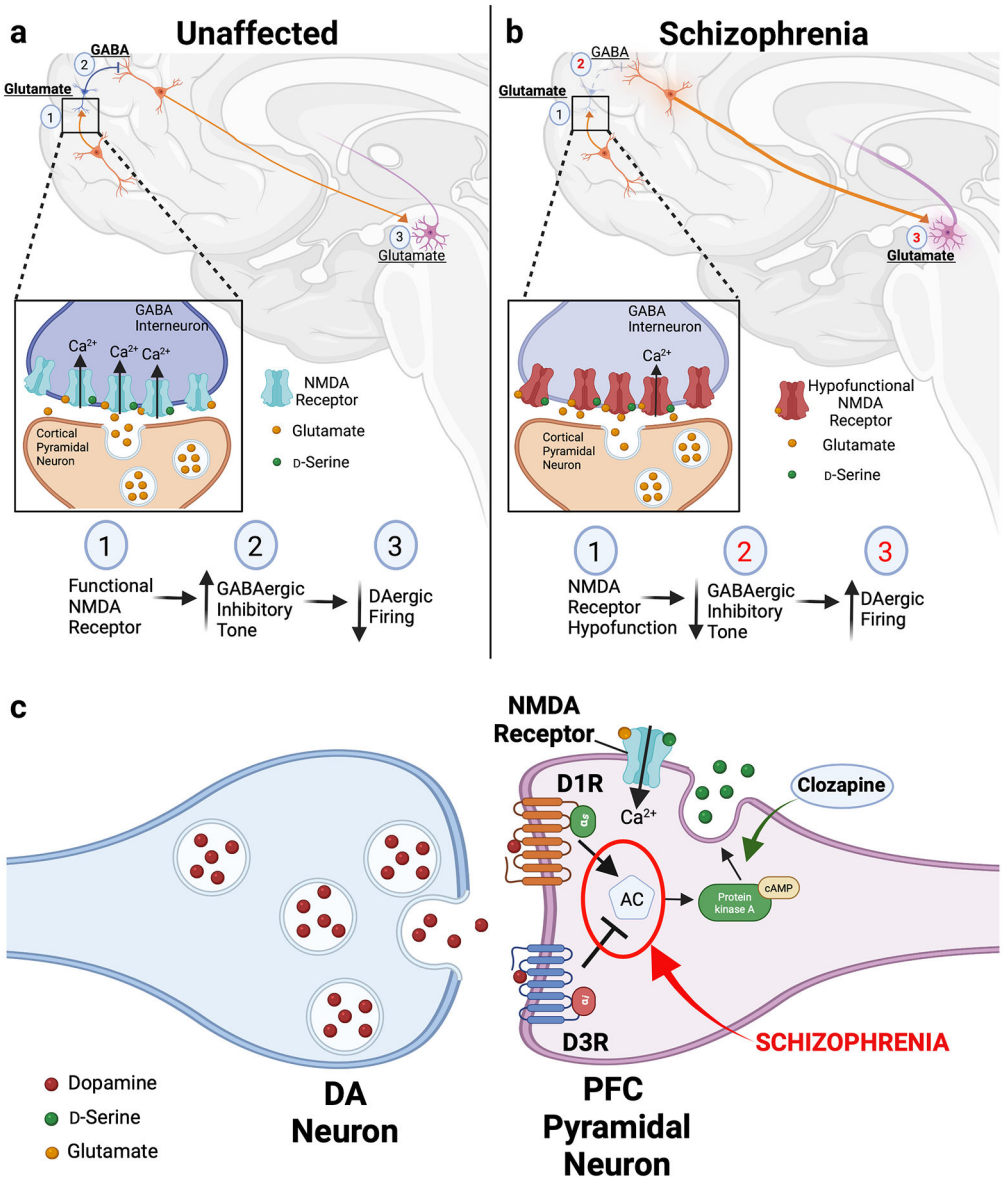

Figure 1. Interplay between dopamine and glutamate systems is disrupted in schizophrenia.

(a) In unaffected individuals, striatal dopamine (DA) levels are modulated via a polysynaptic circuit: (1) Cortical pyramidal glutamatergic projections stimulate GABAergic interneurons via stimulatory NMDA receptors. Upon activation by local increases in synaptic glutamate and co-substrate D-serine, the NMDA receptors conduct Ca^{2+}, triggering increased GABAergic interneuron firing (**inset**). (2) GABA released by these interneurons lowers the firing rate of cortical glutamate neurons that project onto DAergic midbrain neurons. (3) The resulting glutamate tone from these stimulatory projections to the midbrain enables homeostatic control over DAergic firing in the mesolimbic pathway to finely modulate striatal DA levels. **(b)** According to the NMDA receptor hypofunction hypothesis, in schizophrenia, disruptions in DA-glutamate communications within this circuit drive abnormal increases in striatal DA to produce schizophrenia pathology. Hypofunctional NMDA receptors expressed by GABAergic interneurons diminish the amount of Ca^{2+} influx

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in response to glutamatergic stimulation (**inset**). This significantly dampens GABAergic inhibitory tone and increases firing by the midbrain-projecting secondary glutamatergic neurons. The resulting increases in midbrain DA neuron activity raise striatal DA levels, contributing to the positive symptoms of schizophrenia. Red numbers indicate pathway steps affected by schizophrenia pathology. **(c)** In pyramidal neurons of the prefrontal cortex (PFC), the combined actions of dopamine (DA) on stimulatory D_1 (D1R) and inhibitory D_3 (D3R) receptors modulates levels the activity of adenylate cyclase, the enzyme responsible for cAMP synthesis. Tight control over intracellular cAMP enables neurons to control release of D-serine and finely tune local glutamatergic neurotransmission. In schizophrenia, we propose that disturbances in coordination between D1R- and D3R-mediated cAMP signaling alter dopaminergic regulation of D-serine release (in red). This consequently produces pathologic changes in the downstream glutamatergic circuitry. Conversely, treatment with the antipsychotic drug clozapine raises D-serine release (in green), providing a potential therapeutic mechanism for ameliorating the disturbances in the dopaminergic modulation of D-serine release.

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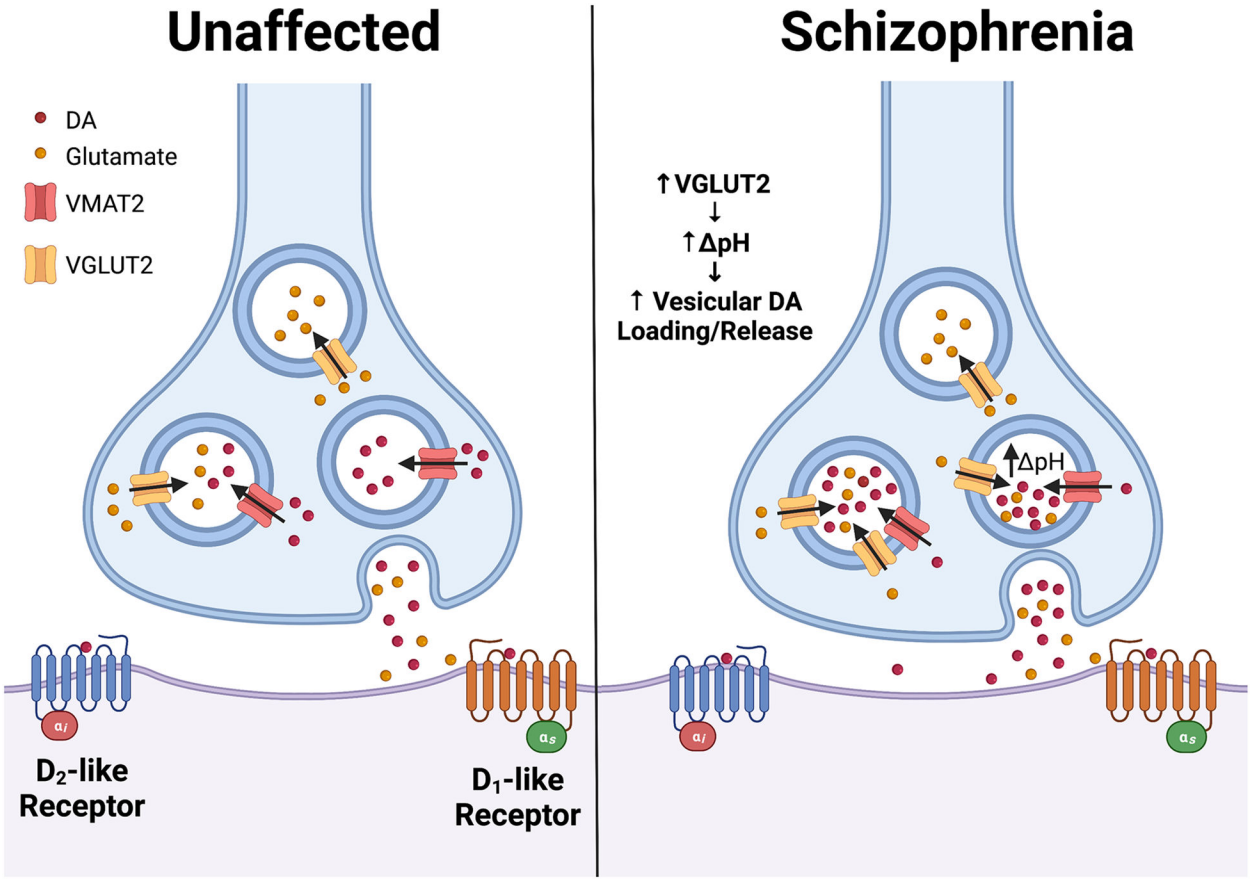

Figure 2. Model for vesicular glutamate transporter 2 (VGLUT2)-mediated role in elevated striatal dopaminergic neurotransmission in schizophrenia.

In addition to its role in loading glutamate into synaptic vesicles, VGLUT2 mediates activity-dependent vesicular hyperacidification in midbrain dopamine (DA) neurons that co-transmit glutamate. The resulting drop in DA vesicle pH increases the vesicular proton gradient (ΔpH), the main driving force for VMAT2-mediated DA vesicle loading. This mechanism confers the ability of neurons to tune vesicular DA loading in response to changes in neuronal activity. In schizophrenia, we propose that abnormal increases in VGLUT2 expression in midbrain DA neurons may raise activity-dependent hyperacidification by increasing ΔpH. The resulting elevation in vesicular DA loading and release boosts striatal DA levels, contributing to schizophrenia pathology including positive symptoms.