

## Review

# Synaptic functions and their disruption in schizophrenia: From clinical evidence to synaptic optogenetics in an animal model

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**Abstract:** The adult human brain consists of approximately a hundred billion neurons, which are connected via synapses. The pattern and strength of the synaptic connections are constantly changing (synaptic plasticity), and these changes are considered to underlie learning, memory, and personality. Many psychiatric disorders have been related to disturbances in synaptogenesis and subsequent plasticity. In this review, we summarize findings of synaptic disturbance and its involvement in the pathogenesis and/or pathophysiology of psychiatric disorders. We will focus on schizophrenia, because this condition has a high proven heritability, which offers more unambiguous insights into the biological origins of not only schizophrenia but also related psychiatric disorders. To demonstrate the involvement of synaptopathy in psychiatric disorders, we discuss what knowledge is missing at the circuits level, and what new technologies are needed to achieve a comprehensive understanding of synaptopathy in psychiatric disorders.

**Keywords:** synaptopathy, schizophrenia, synaptic integration, optogenetics, AS-PaRac1

## Introduction

Human brain development is a protracted process that begins in the prenatal period and extends into late adolescence. Alterations in gene expression induced by changes in environmental input support ongoing events that define brain development, and disruption of either can fundamen-

tally alter neural outcomes. Epidemiological, developmental and neuroimaging studies have indicated that schizophrenia (SZ) has its origins in disturbances in development (the neurodevelopmental hypothesis of SZ).<sup>1)</sup> Although SZ onset usually occurs in early adulthood, longitudinal research has shown that subtle symptoms, such as deficits in social communication skill and cognition, are generally already existent during childhood. This premorbid period is followed by the prodromal period when mild psychotic symptoms arise. Prodromal individuals are often adolescents experiencing mild disturbances in perception, cognition, language, and social function. Clinicians are aware that there is clear functional deterioration in the individuals who convert from the prodromal state to the full-blown psychotic state, suggesting that dynamic changes in neurobiological processes drive this conversion. One of the most marked changes in this period is synapse reorganization, which was suggested as a contributory factor for SZ.

Electrical events called “action potentials” cause the neuron to release a chemical neurotransmitter into a small space, called the synaptic cleft, between it and a neighboring (postsynaptic) neuron. The released neurotransmitters bind to receptors on the

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Abbreviations: AMPA-R: AMPA-type glutamate receptor; APP: amyloid precursor protein; CaMKII: Ca<sup>2+</sup>/calmodulin-dependent protein kinase II; DISC1: *Disrupted-in-Schizophrenia 1*; ENU: N-ethyl-N-nitrosourea; EPSP: excitatory postsynaptic potential; ERP: event-related potential; IPSP: inhibitory postsynaptic potential; NMDA-R: N-methyl D-aspartate receptor; P30: postnatal day 30; P60: postnatal day 60; PAK: p21-activated kinase; PCP: phencyclidine; PFC: prefrontal cortex; PPI: prepulse inhibition; PSD: postsynaptic density; shRNA: short hairpin RNA; SZ: schizophrenia; VAMP2: vesicle associated membrane protein 2.

postsynaptic neuron, which then contributes to the generation of an action potential in the neuron. It is estimated that a single neuron can have 1,000–15,000 synaptic connections, from which the summation of inputs decides whether an action potential will be generated or not. In this way, the function of a neuron is to integrate information from other neurons and decide whether the processed information should be conveyed other neurons. Thus, neurons are considered to be the computational units of brain function.<sup>2)</sup>

Various lines of clinical evidence, including human genetics, human brain imaging, and post-mortem brain studies, suggest that disturbances in neuronal connectivity (synaptopathy) underlie a variety of psychiatric disorders.<sup>3)</sup> In this review, we summarize what is known about synaptopathy and its involvement in psychiatric disorders. We focused on SZ, because it has one of the highest estimated heritabilities of all psychiatric disorders; therefore, it can give more unambiguous insights into the biological origins of not only SZ but also related psychiatric disorders. Furthermore, we introduce a new technology, “synaptic optogenetics,” which was designed to determine how the biological features of affected circuits account for the specific symptoms of psychiatric disorders.

### Synapses and their function

Synapses can be excitatory or inhibitory, depending on whether they activate or inhibit the postsynaptic neuron.<sup>4),5)</sup> When a presynaptic neuron releases excitatory neurotransmitters (such as glutamate) into the synaptic cleft, some of them bind to ion channels that open to allow an influx of positively charged ions, which generates an excitatory postsynaptic potential (EPSP) (Fig. 1A). Conversely, the entry of negatively charged ions into a postsynaptic neuron causes an inhibitory postsynaptic potential (IPSP) (Fig. 1A). EPSPs and IPSPs generate graded potentials in postsynaptic neurons. The combined effect of multiple EPSPs elicited by synchronous excitatory inputs causes depolarization of the membrane; if this depolarization reaches the action potential threshold, the neuron will fire.<sup>6)</sup> By contrast, IPSPs counter the effects of an excitatory neurotransmitter, making the postsynaptic neuron less likely to fire.

Axons can synapse at various neuron sites, which can have distinct effects on the generation of an action potential (Fig. 1B).<sup>2),7)</sup> Axosomatic synapses, whereby an axon directly synapses at the soma, can lead to direct potential transfer, resulting in

effective modulation of the excitability of the postsynaptic neuron. Axoaxonic synapses, whereby an axon synapses with another axon, can modulate neuronal transmission by either inhibiting or facilitating depolarization. Synapses between two dendrites of different neurons are called dendrodendritic synapses and mediate lateral recurrent and lateral inhibition that causes reciprocal effects on sensory processing in the olfactory bulb and retina.<sup>8),9)</sup> Although the variety of synaptic contacts underlies the complex structure in the brain, the most common are axodendritic synapses, which are those between an axon and a dendrite. In the mammalian cerebral cortex, approximately 80% of excitatory axodendritic synapses are formed on the small protrusions of a neuron’s dendrite (dendritic spines).<sup>10)</sup> This review focuses specifically on the dendritic spines.

The advent of newly developed techniques has revealed a correlation between spine size and the efficacy of synaptic transmission (Fig. 1C). For example, electron microscopic studies have demonstrated a positive correlation between the volume of a spine and postsynaptic density (PSD).<sup>11)</sup> The PSD is a large protein complex that anchors a diverse variety of neurotransmitter receptors, channels, adhesion molecules, and scaffold proteins.<sup>12),13)</sup> Spine volume also positively correlates with the expression of functional AMPA-type glutamate receptors (AMPA-Rs),<sup>14)</sup> which are ionotropic glutamate transmembrane receptors for fast synaptic transmission. Single spine stimulation by two-photon activation (uncaging) of glutamate has revealed that the uncaging-evoked AMPA-R current through the stimulated spine is strongly correlated with spine head size, which supports the hypothesis that dendritic spine size is a key determinant of synaptic efficacy.<sup>14)</sup> Crucially, dendritic spines are highly dynamic and undergo structural enlargement and shrinkage (Fig. 1C).<sup>15)–17)</sup> Single spine long-term potentiation protocols have clearly revealed a strong correlation between the extent of potentiation of AMPA currents and that of spine head enlargement.<sup>16)</sup> As mentioned above, the generation of an action potential is determined by the summation of synaptic inputs, and modulation of synaptic (de)potentiation provides a scalar value that determines what neurons in a circuit will be preferentially activated in response to upstream neuronal activation (Fig. 1D).<sup>18)</sup> Thus, synapses are considered as the basic units of neuronal circuits, which ultimately shape our brains as well as perception, cognition, and behavior.

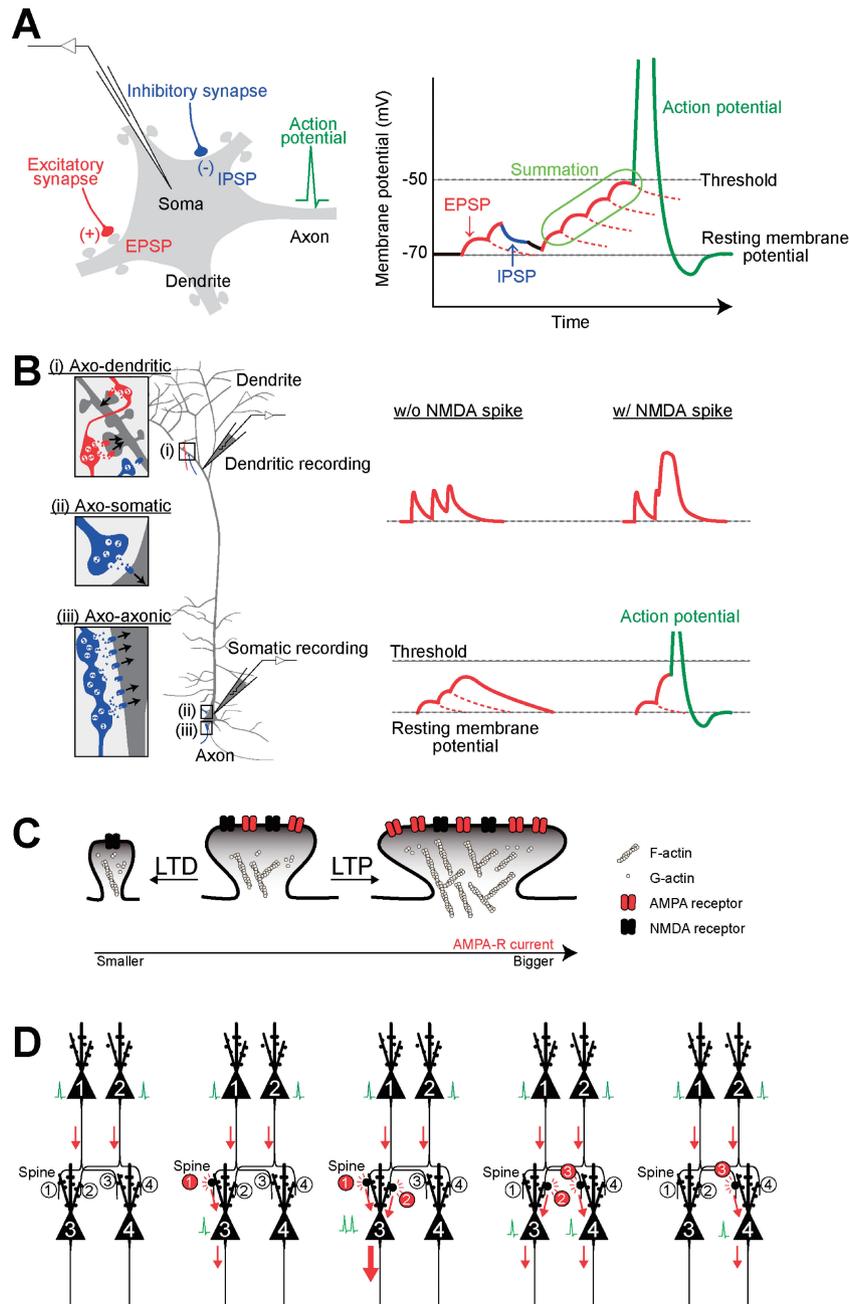


Fig. 1. Effect of EPSP and IPSP on the generation of an action potential. (A) The EPSP/IPSP signal is propagated down the dendrite and is summed with other synaptic inputs at the soma. A single EPSP cannot depolarize the membrane to generate an action potential. However, because EPSPs/IPSPs are graded, they have an additive or subtractive effect on the membrane potential. When the summation of EPSPs/IPSPs reaches the threshold, an action potential occurs. (B) Types of synapses. Axodendritic synapse in tuft dendrites generates an NMDA conductance, which can support a regenerative membrane potential (spike). The NMDA spike has a significant influence on the generation of action potentials. (C) Schematic of long-term potentiation (LTP) and long-term depression (LTD). Upon specific patterns of synaptic activity, the dendritic spine undergoes a marked structural change, which is accompanied by a dynamic change in surface AMPA receptors in the postsynaptic density. The bigger the spine head is, the more AMPA receptors are present. (D) The synaptic weights determined by (de)potentiation provide a scalar quantity of which circuit can be preferentially retrieved in response to the activation of upstream cell ensembles. When Spine #1 is potentiated, the probability of an action potential in Neuron #3 will increase. When both Spine #1 and Spine #2 are potentiated, Neuron #3 is more likely to fire as a part of the cell ensemble. By contrast, when Spine #3 is potentiated, and Spine #1 is depotentiated, Neuron #4, instead of Neuron #3, is more likely to fire.

## Schizophrenia

SZ is a serious mental illness, and because of its early onset, severity, and chronicity, it is considered the most devastating of all psychiatric illnesses.<sup>19)</sup> The term “schizophrenia” is derived from the Greek words “*schizo*” (split) and “*phren*” (mind), which succinctly summarizes the dissociation of thought and cognition observed in SZ patients, which results in delusions, hallucinations, and disorganized and unusual thoughts.<sup>20)</sup> Hallucinations are perceptual experiences that occur independently of an external source; most individuals with SZ experience auditory hallucinations, typically in the form of distressing voices. These include hearing internal thoughts spoken aloud, and a voice that refers to the patient in the third person and in the form of critical commentary. A delusion is a false and irrational belief that a patient holds with a strong conviction, which is not based on evidence and cannot be corrected by reason. One commonality between hallucinations and delusions is the inability to distinguish between something real (processing of external stimuli) and not real (self-generated internal information).

Antipsychotics such as dopamine D2 receptor antagonists have a relatively good efficacy in the reduction of hallucinations and delusions.<sup>21)</sup> However, it is estimated that up to 60% of patients with SZ experience medication-resistant hallucinations and delusions.<sup>22)</sup> Indeed, clinicians are aware that some hallucinations in SZ patients are persistent, and typically manifest as repetitions of the same phrases in the same tone. The robustness of these residual symptoms suggests that a specific set of neurons (a psychosis-related neuronal ensemble) underlying impaired information processing are also robustly formed and stably activated. It remains unknown as to how such an ensemble can be spontaneously and stably activated. However, given that the summation of synaptic inputs largely determines whether the corresponding neuron fires or not (Fig. 1A), it is conceivable that improper integration of synaptic inputs can elicit sensory-irrelevant firing of the psychosis-related neuronal ensemble. Action potentials are not elicited by “synaptic democracy,” whereby all synapses of a neuron have the same influence on the somatic membrane potential. In reality, altered distribution of excitatory and inhibitory synapses within a neuron produces stably activated synaptic connections that have a dominant effect on action potential generation

through active dendritic processing.<sup>2),23)</sup> In the following section, we will review pathological findings of synapses in patients with SZ and discuss how this might disturb synaptic integration and eventually result in sensory-irrelevant firing.

### Postmortem brain studies and functional brain imaging of schizophrenia

One of the most consistently observed neuropathological alterations in postmortem brains of patients with SZ is the reduction in dendritic spine density (Table 1).<sup>24)–30)</sup> Because the majority of excitatory synapses in the cerebral cortex are formed on the spine, and spine size strongly correlates with its synaptic efficacy, the evaluation of dendritic spines is straightforward to assess synaptic function in postmortem brains. Two independent research groups have conducted careful comparisons between SZ brains and control brains, in which case and control brains were matched for sex, age, smoking/alcohol intake, postmortem interval, and agonal states that can significantly affect the antemortem structure of the brain. Both research groups have consistently reported that spine density was significantly reduced in layer III pyramidal neurons of the prefrontal cortex (PFC) in SZ brains. This reduction in spine density was not observed in layer V or VI, nor in layer III of the primary visual cortex, which suggests that the synaptic pathology of SZ takes place in specific neuronal circuits, and not throughout the entire brain (Table 1). This decrease in spine density is likely to be a result of the illness itself rather than the antipsychotics, since these drugs do not reduce dendritic spine density,<sup>31)</sup> number of neurons,<sup>32)</sup> or neural markers.<sup>33)</sup> A decrease in the immunoreactivity of a postsynaptic marker, Spino-philin, has repeatedly been reported, providing complementary evidence for the reduction in spine density in the primary auditory cortex.<sup>28),30)</sup> The decrease in spine number is only seen for smaller spines ( $<0.15\ \mu\text{m}^3$ ), not for larger spines.<sup>30)</sup> The recent advent of within-subject two-photon spine imaging of rodents has revealed that large spines are, in general, highly stable, whereas small spines are more plastic and related to learning and behavioral flexibility.<sup>16),34)</sup> Mechanistic insights into whether this might form the basis of altered circuitry in SZ are currently lacking. The loss of the small spines might contribute to disease-related circuitry, in that this would inevitably result in less plastic circuits. Alternatively, loss of small spines might disrupt the balance in dendritic computations.

Table 1. Postmortem studies in schizophrenia. Findings concerning dendritic spines in schizophrenia

1st author	Year	Journal	Method	Brain regions				Cell type	Dendrite type	Findings
				Frontal	Temporal	Visual	Hippocampus			
Garey LJ <sup>24)</sup>	1998	J Neurol Neurosurg Psychiatry	Golgi	10, 11, 45	11, 20, 21, 22, 38	n.d.	n.d.	Layer III pyramidal neuron	All the dendrites	66% decrease in frontal regions. 59% decrease in temporal regions.
Glantz LA <sup>25)</sup>	2000	Arch Gen Psychi	Golgi	46	n.d.	17	n.d.	Layer III pyramidal neuron (Superficial and deep layer)	Basal dendrites	21% decrease in the deep layer of DLPFC. No change in the superficial layer of DLPFC. No change in the primary visual cortex.
Rosoklija G <sup>26)</sup>	2000	Arch Gen Psychi	Golgi	n.d.	n.d.	n.d.	Subiculum	Pyramidal neuron	Apical dendrites	72% decrease in subiculum.
Kolluri N <sup>27)</sup>	2005	AM J Psychi	Golgi	46	n.d.	n.d.	n.d.	Layers V/VI pyramidal neuron	Basal dendrites	No change in layers V and VI.
Sweet RA <sup>28)</sup>	2009	Neuropsychopharmacol	Spinophilin puncta	n.d.	41, 42	n.d.	n.d.	Layer III pyramidal neuron (Deep layer)	All the dendrites	27% decrease in deep layer III of BA41. 22% decrease in deep layer III of BA42.
Konopaske GT <sup>29)</sup>	2014	JAMA Psychi	Golgi	46	n.d.	n.d.	n.d.	Layer III pyramidal neuron (Deep layer)	Basal dendrites	7% decrease in deep layer III of BA46.
MacDonald ML <sup>30)</sup>	2017	AM J Psychi	Spinophilin puncta	n.d.	41	n.d.	n.d.	Layer III pyramidal neuron (Deep layer)	All the dendrites	27% decrease only for smaller spine.

The contributory effect of synaptopathy on SZ symptoms has been further demonstrated by a study of the N-methyl D-aspartate receptor (NMDA-R).<sup>35)</sup> A within-subject analysis revealed a highly significant correlation between antemortem cognitive deterioration and *NR1* (a subunit of NMDA-R) mRNA loss in the postmortem temporal cortex (BA 22) of patients with SZ.<sup>36)</sup> The expression level of *NR1* mRNA does not correlate with the age or chronicity of the disorder,<sup>36)</sup> which suggests that the decrease in *NR1* mRNA may not be due to an atrophic change in neural circuitry resulting from prolonged hospitalization or long-term exposure to antipsychotics. Perhaps the most compelling evidence for NMDA-R dysfunction in SZ is that

administration of a noncompetitive NMDA-R antagonist, phencyclidine (PCP), induces a broad range of SZ-like symptoms.<sup>37),38)</sup> Many drugs can cause hallucinations and delusions, but the ability of PCP to mirror almost all aspects of the symptomatology of SZ is unparalleled, and even experienced psychiatrists sometimes misdiagnose chronic PCP abuse as SZ. Chronic PCP treatment in rats and non-human primates also mimics SZ-related behavioral alterations, such as working memory deficits and deficits in PFC-dependent tasks.<sup>39)–42)</sup> The administration of PCP in rats causes an initial hyperactivity of cortical areas followed by a delayed depression of activity.<sup>43)</sup> Given that PCP is an NMDA-R antagonist, PCP-induced transient cortical hyperexcitation sounds

paradoxical. The currently accepted explanation is that PCP receptor affinity differs depending on cell type. PCP is an open-channel blocker and there are more open (active) NMDA-Rs on fast-spiking GABAergic cells than on slow-spiking cells such as excitatory pyramidal neurons. Thus, administration of PCP preferentially suppresses the activation of these inhibitory neurons, resulting in a dramatic disinhibition of pyramidal neuron activity and elevated uncoordinated firing throughout the cortico-limbo-thalamic circuit.<sup>44)</sup>

Another line of evidence is derived from the discovery of anti-NMDA-R encephalitis, which resembles the severe psychotic symptoms of SZ (hallucinations and delusion).<sup>45)</sup> This disease has generated tremendous interest because of its unambiguous etiology; it has been classified as a subtype of SZ that can be readily identified and treated. Studies on anti-NMDA-R encephalitis have revealed that anti-NMDA-R IgG recognizes the NR1 subunit of NMDA-R, which results in an internalization of the receptors from both the synaptic and extrasynaptic space in both excitatory and inhibitory neurons.<sup>46)</sup> As a result, an imbalance of excitation/inhibition could result in the increased excitability of pyramidal neurons. The relative increase in glutamatergic transmission in anti-NMDA-R encephalitis is reminiscent of the results of proton magnetic resonance spectroscopy studies of patients with general SZ, which showed elevated glutamate levels in first-episode, drug-naïve patients, and a decrease in glutamate levels after treatment.<sup>47)</sup> Taken together, this evidence indicates that dysfunction of NMDA-R signaling is related to the degree of cognitive decline in SZ.

How do NMDA-Rs affect neuronal transmission within cortical circuits? While the AMPA-R permits  $\text{Na}^+$  and  $\text{K}^+$  influx to mediate basic synaptic transmission, the NMDA-R has some distinct features. First, the NMDA-R pore is blocked by  $\text{Mg}^{2+}$  at voltages near the resting membrane potential. The postsynaptic cell membrane depolarizes as  $\text{Na}^+$  and  $\text{K}^+$  ions enter the cell via AMPA-Rs, eventually resulting in sufficient depolarization, which in turn relieves the voltage-dependent  $\text{Mg}^{2+}$  block of NMDA-Rs. Thus, the NMDA-R functions as a coincidence detector of simultaneous activation of a presynaptic and a postsynaptic neuron.<sup>2)</sup> Second, once the  $\text{Mg}^{2+}$  block is relieved, the NMDA-R is permeable to  $\text{Ca}^{2+}$  in addition to  $\text{Na}^+$  and  $\text{K}^+$  ions.  $\text{Ca}^{2+}$  acts as a second messenger and activates various calcium-dependent proteins, including calmodulin, calcineurin, protein kinase C, and  $\text{Ca}^{2+}$ /calmodulin-dependent protein

kinase II (CaMKII), all of which are crucial for synaptic plasticity.<sup>48),49)</sup> Third, simultaneous activation of NMDA-Rs across multiple synapses in close spatial proximity along a dendritic segment can generate a non-linear effect on the local potential, termed “NMDA spikes” (Fig. 1B).<sup>50),51)</sup> This local NMDA-R-dependent potential, a highly supralinear summation of multiple inputs, has a much more significant impact on the generation of an action potential than the summation of the separate effects, and enhances the generation of action potentials at the soma. This could explain why NMDA-Rs play a vital role in the integrative properties of pyramidal neurons, which are not simple relay neurons but also process information via active dendritic computation.<sup>52)</sup> Moreover, two-photon uncaging of glutamate can induce spinogenesis in cortical layer II/III pyramidal neurons during the early postnatal period.<sup>53)</sup> Preventing NMDA-R activation with an NMDA-R antagonist (CPP) abolishes spinogenesis, whereas an AMPA-R antagonist (NBQX) has no effect, suggesting that NMDA-R can influence the capacity or threshold for excitatory synaptic connections during the early postnatal period. Given the unique biophysical properties of the NMDA-R, NMDA-R-mediated signaling emerges as a powerful mechanism for neuronal network formation during development and later for plasticity via its ability to fine-tune information processing, encoding, and storage throughout the brain.

Another critical molecule that regulates neuronal firing is the inhibitory neurotransmitter GABA. A variety of deficits in GABAergic markers in the PFC have been reported in postmortem SZ brain studies, which has indicated that SZ is associated with lower inhibitory transmission.<sup>54)–56)</sup> One of the principal roles of GABA in the inhibition of neuronal excitability is to synchronize neuronal networks, which generates rhythmic or bursting neural activity, termed oscillations.<sup>57)</sup> A persistent oscillatory firing of a context-specific neural ensemble in the PFC enables distinct information to be linked across time and space (different brain regions), which results in the integration of sensory and mnemonic information; in turn, this integrates and manipulates multiple representations.<sup>58)</sup> Thus, proper oscillatory firing in the PFC is believed to serve as a cellular mechanism of working memory. Considering that working memory deficits have been proposed to underlie a broad range of cognitive deficits in SZ patients,<sup>59)</sup> disruption of PFC oscillation could be involved in SZ. A decrease in the expression of the

GABA-synthesizing enzyme GAD67 and a deficit in parvalbumin-positive GABAergic interneurons are among the most robust findings from postmortem SZ brains.<sup>60)</sup> The fact that the expression of both GAD67 and parvalbumin is activity-dependent further indicates that GABAergic drive is reduced in the PFC in SZ. Thus, it is clear that both transmissions are altered in SZ; however, it is not yet known which alterations are etiologic and which are downstream consequences of the disease. Compelling evidence indicates that genetics has a substantial influence on the etiologies of SZ. Thus, the next section will review the genetic basis of SZ, and will attempt to narrow down its causal pathology.

### Genetic studies of schizophrenia

Family, twin, and adoption studies support the strong involvement of genetic factors in the pathogenesis of SZ.<sup>61),62)</sup> Despite the 0.7% lifetime morbid risk of SZ in the general population, the largest twin cohort study as of 2017 estimated that genetic factors may explain as much as 79% of SZ risk.<sup>63)</sup> The “Genain Quadruplets,” a set of monozygotic female quadruplets who all developed SZ, demonstrate this significant genetic component.<sup>64)</sup> Thousands of genetic studies of SZ, including genetic linkage, association studies, genome-wide association studies, and whole exome sequencing studies, have identified numerous SZ susceptibility genes.<sup>65)</sup> Nonetheless, no single gene *per se* crucial for the generation of SZ has been reported. Instead, the accumulation of SZ susceptibility gene products seems to operate synergistically in the same biological pathway. Thus, a reasonable next step would be to clarify which biological pathways are affected by these genetic variations. A comprehensive review of SZ genetics is not within the scope of this review but can be found elsewhere.<sup>1)</sup> In short, Gene Ontology analysis has identified several potentially affected pathways involved in synaptic transmission (*HTR2A*, *BDNF*, *CHRNA3*, *CHRN4*, *DRD1*, *DRD2*, *DRD4*, *EGR1*, *GRIA4*, *GRIN2B*, *GRM3*, *NISCH*, *SLC1A3*, and *ERBB4*), dopamine metabolic processes (*COMT*, *DRD1*, *DRD2*, *DRD4*, *MAOA*, *NR4A2*, and *SLC6A3*), and ion channel activity (*CHRNA7*, *CACNA1I*, *CACNB2*, *CHRNA3*, *CHRNA5*, *CHRN4*, *GABRB3*, *GRIA4*, *GRIA1*, *GRIN2B*, *HCN1*, *CACNA1C*, *KCTD13*, *KCNV1*, *KCNN3*, *KCNJ13*, and *KCNB1*).<sup>65)</sup> A family-based exome sequencing study to detect *de novo* mutations found that non-synonymous mutations were enriched, particularly in NMDA-R and Activity-Regulated

Cytoskeleton-Associated Protein signaling.<sup>66),67)</sup> Proteins with non-synonymous *de novo* mutations are more likely to bind to each other and connect with synaptic proteins, which suggests that altered synaptic networks may be a causal genetic predisposition for SZ.

These sophisticated genetic studies have revealed that synaptic network mutations are significantly involved in the development of SZ (Fig. 2). However, a genetic association does not provide insights into the cellular mechanism of the disorder. As such, no matter how significantly a genetic variant is involved in SZ, it is still unclear which variant of which gene should be studied to determine which disease-related variant is a mechanistic component of the disease. Thus, large-scale exome studies have been employed to spot rare variants with a strong effect on disease risk. It has been estimated that, in the 2,600 Mendelian diseases for which the genetic component has been identified, approximately 85% of the disease-related variations reside in the protein-coding region or in canonical splice sites.<sup>68)</sup> This is one reason why neuroscientists like to exploit rare variants with strong effect size on disease risk as a model, because the connection to a gene is relatively direct and can be evaluated biologically. A successful example of this approach is the discovery of amyloid precursor protein (APP) and presenilin1/2 involvement in familial forms of Alzheimer’s disease.<sup>69)</sup> Although familial Alzheimer’s disease represents less than 1% of general Alzheimer’s cases, the biological understanding of APP and presenilin has revolutionized this research field, because the pathophysiology of familial Alzheimer’s disease is similar to that of a common sporadic form of Alzheimer’s. Indeed, many transgenic mouse models have successfully mimicked Alzheimer-like amyloid-deposition pathology.<sup>70)</sup> This raises the question of whether this approach is feasible in the SZ research field. Among SZ susceptibility genes, the *Disrupted-in-Schizophrenia 1 (DISC1)* gene is probably the best known and most extensively characterized gene in psychiatric genetics.<sup>71)</sup> *DISC1* was identified at the breakpoint on chromosome 1 of the balanced translocation (1;11)(q42.1;q14.3), and was originally identified in a large Scottish family with a high prevalence of SZ and related psychiatric disorders.<sup>72)</sup> The sequence of the chromosome 1 breakpoint indicates that the translocation directly disrupts the gene,<sup>73)</sup> and *DISC1* transcript levels are reduced to half normal levels in translocation carriers.<sup>74)</sup> By contrast, there has been no evidence

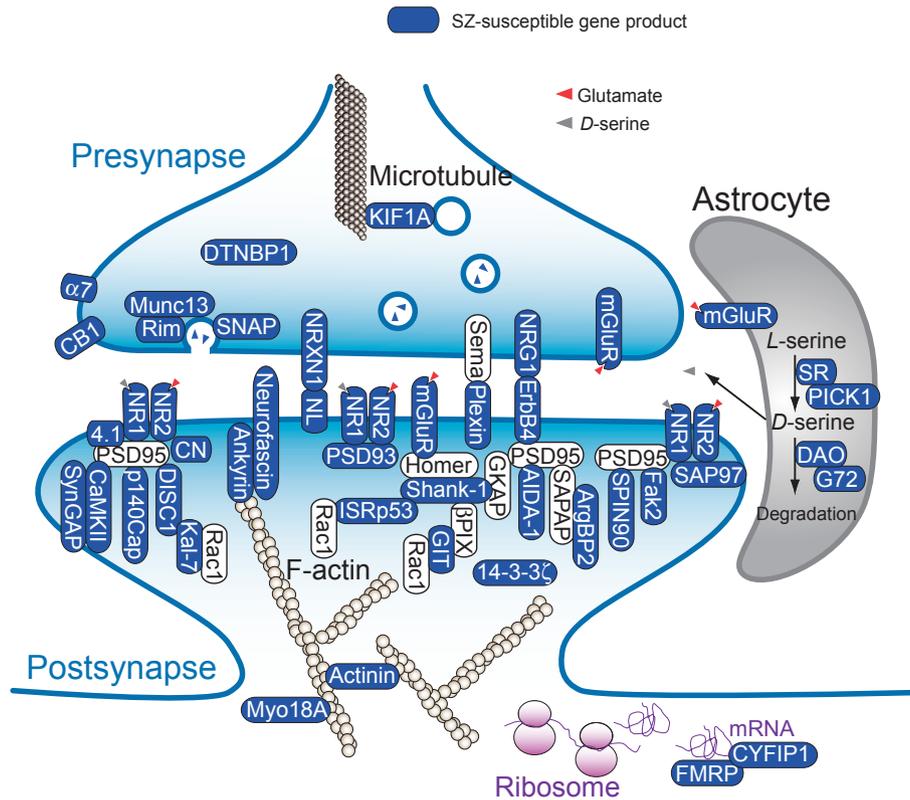


Fig. 2. A synaptic network is a significant schizophrenia-susceptible signal pathway. Schizophrenia-susceptibility genes, which have been repeatedly reported, are depicted as black icons.  $\alpha 7$ , alpha-7 nicotinic receptor; CB1, cannabinoid receptor type 1; CN, Calcineurin; DAO, D-amino acid oxidase; DISC1, Disrupted-In-Schizophrenia 1; DTNBP1, Dysbindin; ErbB4, Erythroblastic leukemia viral oncogene homolog 4; FMRP, fragile X mental retardation protein; G72 (also known as D-amino acid oxidase activator); Kal-7, Kalirin-7; mGluR, metabotropic glutamate receptor; NL, Neuroligin; NR1, NMDA-R, NR1 Subunit; NR2, NMDA-R, NR2 Subunit; NRG1, Neuregulin 1; PICK1, protein kinase C alpha binding protein.

for the presence of any transcripts within the sequence surrounding the chromosome 11 break-point, and no evidence for the presence of a truncated form of the DISC1 protein in t(1;11) translocation cases.<sup>74)</sup> Thus, it is reasonable to assume that the devastating effect of the translocation in the pedigree is due to haploinsufficiency of the *DISC1* gene. The *DISC1* translocation is unique to this single Scottish pedigree, and *DISC1* has not been identified in genome-wide association studies or exome studies, suggesting that *DISC1* itself is not a genetic risk factor for SZ. Nonetheless, enthusiasm for research into DISC1 in the field of SZ has not waned for several reasons. First, multiple members of the Scottish family were affected by SZ or related disorders, and most of the affected individuals were carriers of the translocation. Thus, the t(1;11) translocation has the most substantial effect size in all studies of psychiatric disorders. Second, DISC1 appears to act as a coordinating scaffold protein that

interacts with a huge set of proteins (the DISC1 interactome), which include APP, Dixdc1, LIS1, NDE1, NDEL1 (for neuronal migration),<sup>75),76)</sup> Grb2, Girdin, PDE4B, FEZ1, several RNA-binding proteins (for signaling and trafficking),<sup>74),77),78)</sup> Kalirin-7, and TNK1 (for glutamatergic synapse function).<sup>79),80)</sup> Findings of a positive genetic association (*NDEL1*, *PCMI*, and *PDE4B*) and copy number variation (*NDE1*) have indicated several DISC1-binding proteins to be risk factors for SZ in their own right.<sup>81)</sup> This suggests that the DISC1 interactome pathway is involved in SZ pathophysiology. Thus, despite some controversy about whether the *DISC1* gene is a candidate gene for SZ,<sup>82)</sup> DISC1 biology can be used as a “molecular Rosetta stone” to elucidate the molecular and cellular mechanisms underlying SZ, much like how the rare mutations found in APP and presenilin genes were used to decipher the molecular mechanism of Alzheimer’s disease. In the next section, we will review evidence

from functional studies of individuals with a rare deletion of DISC1 as well as animal models of haploinsufficiency for the *DISC1* gene.

#### Effect of DISC1 disruption by balanced translocation on brain function

The only published study that investigated brain function in t(1;11) carriers measured event-related potentials (ERPs), which are small voltage changes in response to sensory, cognitive, or motor events.<sup>83)</sup> Scalp-recorded ERPs reflect the summation of EPSPs from a large number of consistently oriented cortical pyramidal neurons that fire in synchrony during information processing (Fig. 3A). The P300 is a late ERP component that displays a maximal amplitude over frontal electrodes and has a peak latency in the range of 250–400 ms after stimulus onset. The P300 amplitude is thought to reflect the degree of attention.<sup>84)</sup> The P300 latency can be taken as a measure of speed of information processing, whereby shorter latencies are associated with faster processing and better cognitive performance (Fig. 3B).<sup>85)</sup> Consequently, P300 is an objective, non-invasive measure indicator of information processing, and is valuable for evaluating human brain functioning *in vivo*. Thus, the study employing ERP assessment of t(1;11) carriers is of particular interest because it is the only one to have studied brain function in this pedigree. Regardless of the presence of psychiatric symptoms, the t(1;11) carriers exhibited a significant reduction in the amplitude and latency of the P300 component compared with non-carriers.<sup>83)</sup> This indicates that t(1;11) carriers without symptoms are more similar to individuals with SZ than to normal controls in terms of information processing. This is consistent with the finding that auditory P300 is altered in patients with SZ, as well as their unaffected relatives; therefore, P300 appears to be a marker for the risk of SZ.<sup>83)</sup> P300 reflects brain activity underlying the revision of the mental representations induced by incoming stimuli,<sup>84)</sup> the participant must detect deviant stimuli in a sequence of identical standard stimuli; therefore, this task requires previous events to be held in working memory. The fact that t(1;11) carriers and non-carriers had an identical mean intelligence quotient suggests that DISC1 haploinsufficiency impairs fundamental attention and working memory-related operations but does not cause general brain dysfunction.

To gain a better understanding of SZ disease mechanisms, a mouse model that mimics the Scottish

pedigree was generated. It is not yet clear how accurately mouse models can capture complex human disorders such as SZ. Indeed, a single model cannot be expected to capture all the features of human SZ. Nonetheless, investigations into fear memory in mice have provided critical insights into the etiology of post-traumatic stress disorder in humans, and the essential features of drug addiction in humans can be effectively replicated in rodents. Furthermore, some work has extracted specific mice behaviors, albeit those that are less complex than those of humans, with translational relevance to specific cognitive domains that are affected in SZ. Thus, valuable information can be extracted from mouse models of SZ to identify the behavioral and brain circuitry correlates of the disease. Impairment of working memory and prepulse inhibition (PPI) have received the most attention in the behavioral domain for SZ. There is a diverse repertoire of DISC1-related mouse models, including knockout/knockdown, overexpression of various variants of the *Disc1* gene, and N-ethyl-N-nitrosourea (ENU) mutagenesis in the *Disc1* gene.<sup>86)–91)</sup> Meanwhile, a mouse model of knockout/knockdown that mimics the haploinsufficiency of *Disc1* is only available from three laboratories, and thus we will focus on the model of the haploinsufficiency of *Disc1*.

Kaibuchi and colleagues generated knockout mice for the *Disc1* gene, which lack the full length of *Disc1*.<sup>89)</sup> The knockout mice exhibit various behavioral abnormalities, including elevated anxiety, higher impulsivity, an impairment of sensorimotor gating as indicated by PPI deficits and methamphetamine-induced hyperactivity that is indicative of altered dopaminergic neurotransmission. These mice also show altered synaptic plasticity, including a threshold shift in the induction of long-term potentiation in the dentate gyrus. A second line for this mouse model was generated by the Gogos laboratory, which most closely mimicked Scottish t(1;11) carriers by introducing two termination codons into exons 7 and 8, eliminating the full length *Disc1* protein.<sup>92)</sup> Both homozygote and heterozygote mutant *Disc1* mice showed impairment in learning acquisition as well as in working memory compared with control littermates. These mice also exhibited a significant decrease in the number of spines in granule cells. A third line of this mouse model contain a knockdown of *Disc1* by short hairpin RNA (shRNA).<sup>79),90)</sup> However, some of the results of earlier studies using shRNA should be interpreted with caution because of insufficient control for off-target effects.<sup>78)</sup> None-

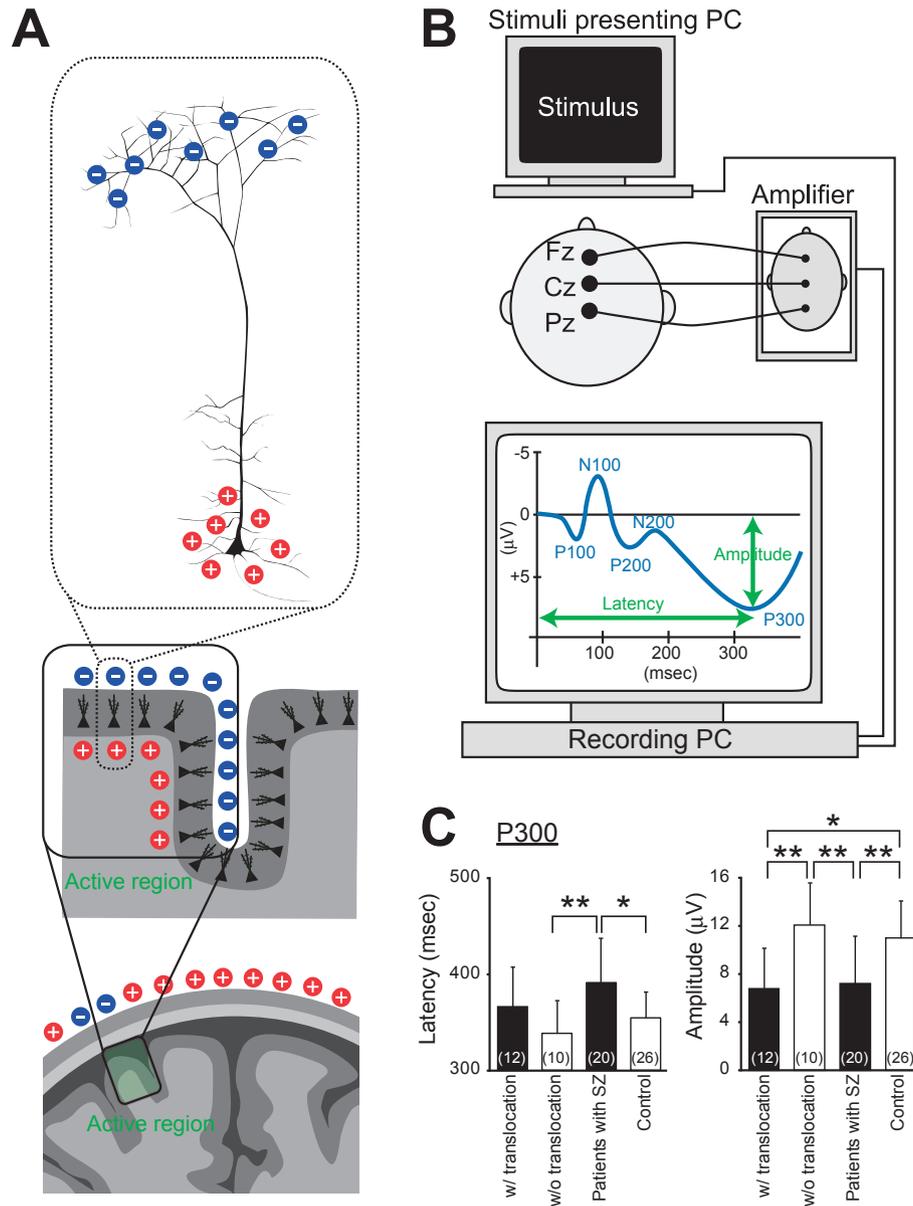


Fig. 3. ERP abnormalities during information processing in schizophrenia. (A) An excitatory transmitter released on apical dendrites causes the discharge of positively charged ions into dendrites, resulting in a net negative charge on the outside of the cell. Pyramidal neurons are mostly oriented in the same direction relative to the cortical surface. Thus, scalp-recorded ERPs are the result of summated EPSPs and IPSPs in layered structures that have a consistent orientation, such as that of vertically oriented pyramidal cells of the cerebral cortex. (B) Schematic of the ERP experiment. (C) P300 latency and P300 amplitude in a Scottish family with the balanced chromosomal t(1;11) (q42.1;q14.3) translocation, in patients with schizophrenia, and in controls. The graph was drawn from original data by Blackwood and colleagues.<sup>83</sup> Error bars are the standard deviation of the mean. Multivariate analysis of variance. Scheffé's *post hoc* comparisons of means showed significant differences between groups. \* $P < 0.05$ , \*\* $P < 0.01$ .

theless, given that chronic depletion of *Disc1* in the conventional knockout model may induce molecular compensation during development, the acute knockdown effect by shRNA provides additional evidence of *Disc1* biology. We previously investigated the effect of shRNA against the *Disc1* gene in Sprague-

Dawley rats<sup>79</sup>) and C57BL6/J mice<sup>90</sup>) and revealed that *Disc1* knockdown induces a robust reduction in spine density in primary cortical neuron cultures. *In vivo* two-photon spine imaging revealed a progressive decrease in spine density in the pyramidal neurons of layer II/III of the PFC when targeted by

*Disc1* shRNA (Fig. 4A). Biochemical analyses have also revealed that *Disc1* knockdown leads to Rac1 activation, which was detected by increased GTP-bound Rac1 and phosphorylation of p21-activated kinase (PAK1).<sup>79)</sup> PAK is a well-characterized kinase and a druggable target, with FRAX486 established as a PAK1 inhibitor. FRAX486 was injected intraperitoneally into *Disc1* knockdown C57BL/6J mice from postnatal days 30 (P30) to 60 (P60) (Fig. 4B). A longitudinal spine analysis by *in vivo* two-photon imaging demonstrated that FRAX486 prevented progressive spine deterioration in *Disc1* knockdown mice (Fig. 4C, D) and partially normalized the impairment of PPI at P60 (Fig. 4E).<sup>90)</sup>

#### What remains unknown about schizophrenia

Thus far, we have summarized the significant findings in SZ research. One of the most important questions is whether these results are causal or whether they instead reflect a compensatory secondary consequence. Although genetic studies indicate a causal role of the synaptic network, mechanistic insights into the molecular and cellular pathophysiology of SZ remain to be elucidated. Dopaminergic hyperexcitation is likely to account only for positive symptoms such as hallucinations and delusion, and this is based on the following reasons: first, dopaminergic psychostimulants provide a good model of the positive symptoms of SZ, but they do not accurately mimic the cognitive or negative symptoms (blunting of affect, apathy, anhedonia, and reduced social drive).<sup>93)</sup> Second, most antipsychotics are dopamine D2 antagonists, and these drugs have limited efficacy for treating negative and cognitive symptoms.<sup>94)</sup> Regarding dopaminergic transmission, brain imaging studies suggest that SZ results in dopaminergic hyperactivation in subcortical regions (mesoaccumbens circuit) and hypoactivity in the mesoprefrontal circuitry.<sup>95)</sup> Mesoprefrontal dopaminergic neurons are directly activated by PFC efferents, which are dysregulated in SZ,<sup>96)</sup> and this results in reduced cortical dopaminergic transmission. In contrast, mesoaccumbens dopaminergic neurons are under an indirect inhibitory regulation by the PFC, which supports the hypothesis that dysregulated PFC outflow in SZ leads secondarily to excessive dopaminergic transmission, which subsequently elicits positive symptoms. Thus, glutamatergic and GABAergic connectivity within the PFC has received much attention in the context of SZ, particularly concerning cognitive function.<sup>97)</sup>

Although a correlation between synaptic pathology and behavioral deficits has been repeatedly demonstrated, this does not necessarily mean that these features are causally related. To identify synaptic abnormalities directly that could account for psychiatric pathology, new methods have been developed (see below).

#### Synaptic optogenetics for synapse visualization and manipulation

Optogenetics is a recently developed method that uses light to manipulate molecular and cellular events in a targeted cell of living animals.<sup>98)–100)</sup> This method is a fruitful technological fusion of optics and genetic engineering, which maximizes the advantages of each discipline.<sup>101)</sup> It allows the millisecond-scale regulation of optical control and specific gene expression and trafficking of the gene product with subcellular precision.<sup>102)</sup> For example, Channelrhodopsin-2, which is a light-activated cation channel that was discovered in the green alga *Chlamydomonas reinhardtii*, enables the control of the membrane potential of targeted neurons, driving it above the action potential threshold. This allows scientists to control neural activity using blue light with millisecond-scale temporal precision. In addition, photo-inhibitory membrane proteins have been engineered.<sup>99),100),103),104)</sup> Therefore, optical activation, silencing, and (de)synchronization of neuronal activity are now all possible; this provides 'necessary and sufficient' evidence to elucidate how neuronal networks work, and how they relate to behavior.

Optogenetics has been extensively applied to study neuropsychiatric disorders such as depression and anxiety in laboratory animals.<sup>105)–110)</sup> Such studies have identified dysfunctional neuronal circuits as an essential part of the pathophysiology of psychiatric disorders. Although this technique is a powerful tool to control an entire cell, it cannot be used to manipulate individual synapses. Given this limitation, a tool that is able to manipulate specific subsets of synapses that are activated in different cell ensembles would allow elucidation of the causal role of synaptic function in behavior. With this in mind, we developed a novel synaptic optoprobe, activated synapse targeting photoactivatable Rac1 (AS-PaRac1), for use in synaptic level photo-manipulation (Synaptic optogenetics) (Fig. 5A).<sup>111)</sup> This optoprobe has two characteristic features. First, AS-PaRac1 can specifically label recently potentiated (enlarged or newly formed) spines (Fig. 5B). Second, AS-PaRac1 induces the selective shrinkage of AS-

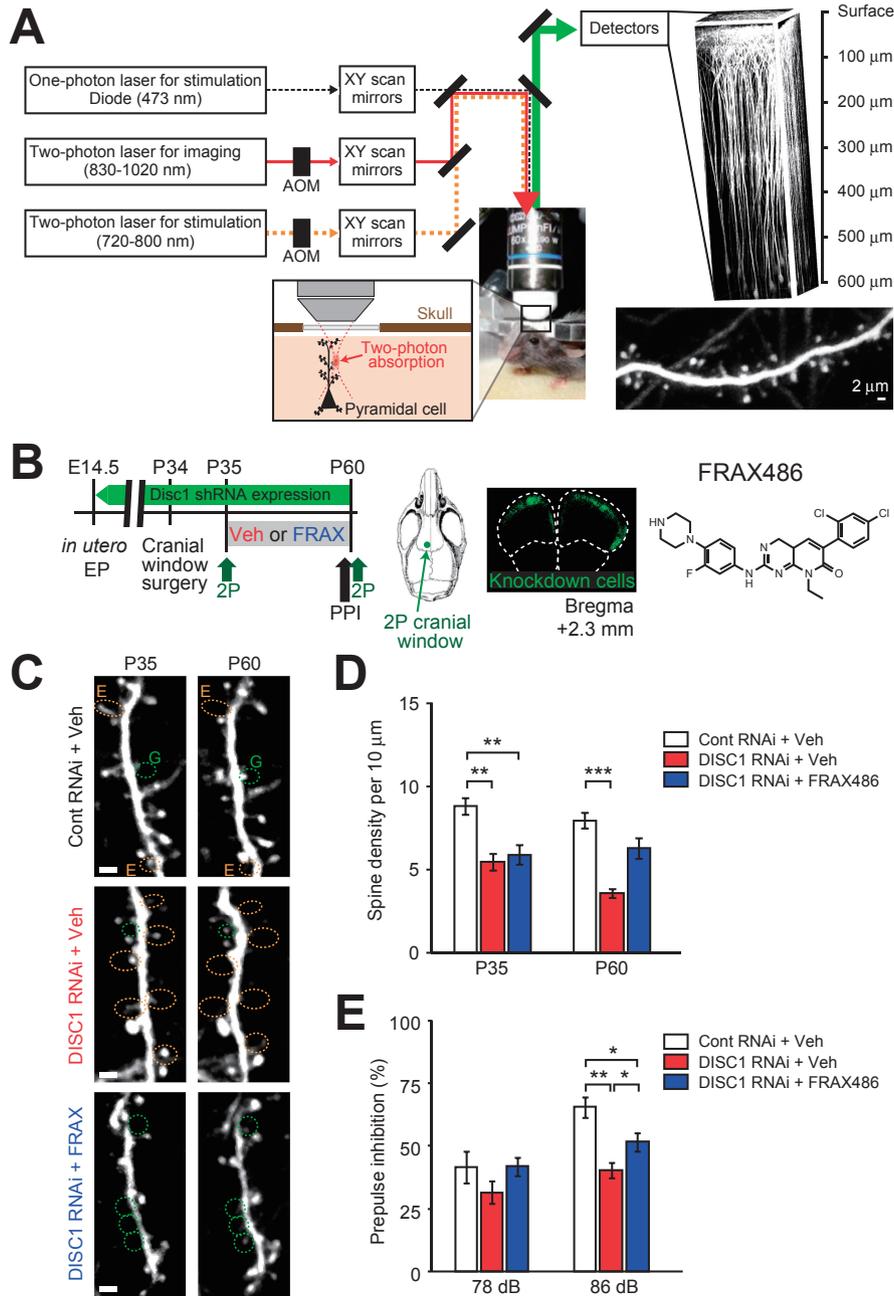


Fig. 4. Progressive synapse loss in a *Disc1* knockdown mouse model. (A) Experimental setup for *in vivo* 2-photon imaging, which allows longitudinal imaging, as well as a specific pattern of photostimulation, in the entire layer of the cerebral cortex. (B) Experimental design. *Disc1* or control shRNA was transferred at embryonic day 14.5 (E14.5). Mice with shRNA were subjected to cranial window surgery at postnatal day 34 (P34). 2-photon imaging was performed both at P35 and P60. Location of the cranial window and *Disc1* knockdown cells (in green) at P60 are shown. Chemical structure of the PAK inhibitor (FRAX486) is shown. Prepulse inhibition (PPI) was tested just before two-photon imaging at P60. (C) Representative spine morphology under each condition. (D) *Disc1* knockdown led to a marked reduction in spine density at both P35 and P60, which was further augmented at P60 (\*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). Daily administration of FRAX486, but not that of vehicle, between P35 and P60, blocked progressive spine loss during adolescence. The effects of FRAX486 were significant in blocking spine elimination (\*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). Spine surrounded by yellow and green dots indicate eliminated (indicated as E) and generated (indicated as G) spines between P35 and P60, respectively. Scale bar, 2 μm. (E) Performance in PPI at P60. The deficit in PPI in *Disc1* knockdown mice was significantly, although only partially, ameliorated by FRAX486 ( $n = 12$ /each group). \* $P < 0.05$ , \*\* $P < 0.01$ . Images in B to E are from Hayashi-Takagi *et al.* (2014)<sup>90</sup> and have been modified with permission.

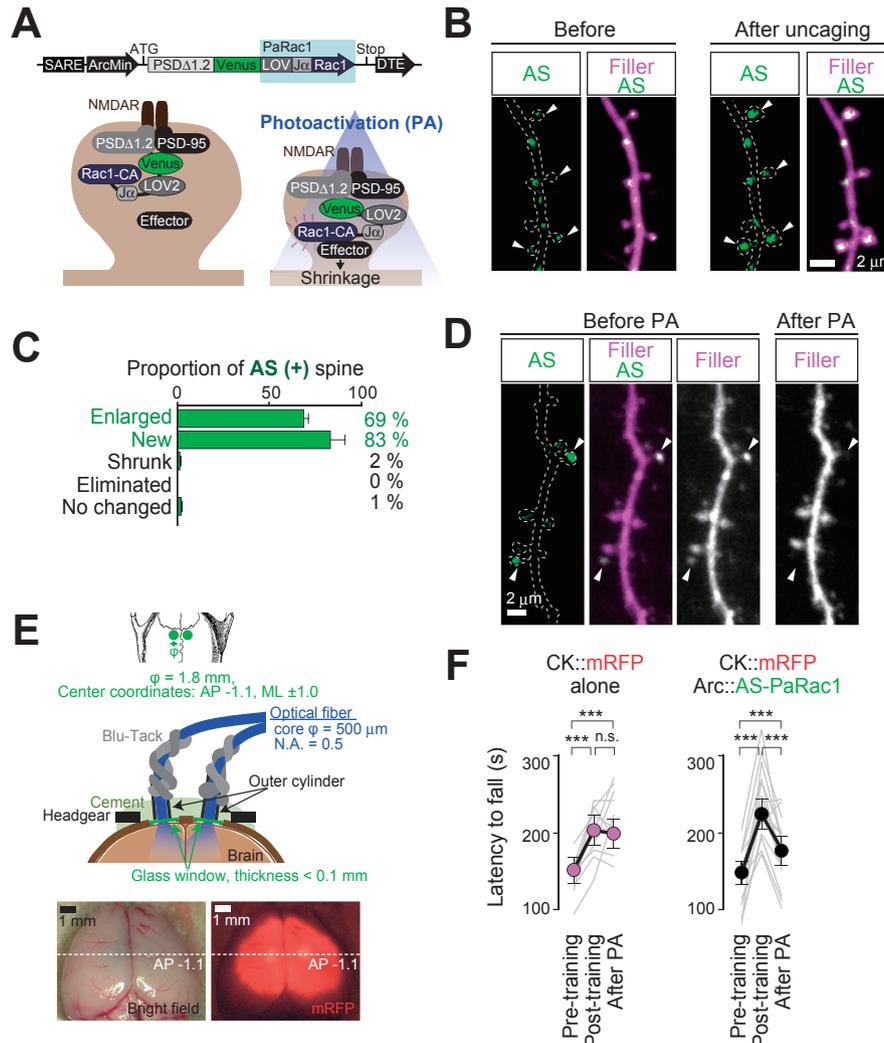


Fig. 5. Optical erasure of acquired skills by synaptic optogenetics. (A) Schematic of the AS-PaRac1 vector, which is transcribed in a synaptic activity-dependent manner. The LOV2 domain was attached to the constitutively active form of Rac1 (Rac1-CA), hindering effector binding of Rac1 until blue light irradiation. Therefore, the blue light can control Rac1-evoked spine shrinkage. (B) The single spine LTP protocol, which was initiated by glutamate uncaging in the presence of the adenylyl cyclase activator forskolin, induced the accumulation of AS-PaRac1 in the stimulated spines (arrowheads) of a hippocampal slice culture (DIV 11). (C) AS-PaRac1 labeling *in vivo* during the rotarod motor task. Percentage of AS-PaRac1-positive spines for enlarged, newly formed, shrunk and eliminated spines in the primary motor cortex upon motor learning. (D) Selective shrinkage of AS-PaRac1-positive spines by photoactivation (PA). Neurons in the hippocampal slice culture (DIV 11) were biologically transfected with AS-PaRac1-Venus and filler. Robust shrinkage (arrowheads) was observed, whereas AS-PaRac1-negative spines were not affected by PA, despite being located adjacent to the AS-PaRac1-positive spines. (E) Schematic of the implantation of optical fibers and AS-PaRac1 transduction in the bilateral primary motor cortex. (F) Erasure of acquired learning by PA of the potentiated spines labeled with AS-PaRac1. The mice were divided into two groups: animals in the first group were infected with CaMKII promoter::mRFP (CK::mRFP) alone as a control, while those in the second group were infected with AS-PaRac1 and mRFP in the primary motor cortex. Both the groups exhibited significantly better motor performance after training (Post-training), but only the performance of the AS-PaRac1 group was disturbed by PA (After PA). In contrast, there was no disruption of acquired learning in the control group. One-way repeated measures ANOVA for the comparison of task performance for the same subjects at different time points followed by *post-hoc* Scheffé's test (\*\*\*)  $P < 0.001$  were used. Image adapted, with permission, from Hayashi-Takagi *et al.* (2015).<sup>111</sup>

PaRac1-containing spines upon blue light irradiation. Thus, this method not only allows us to label potentiated spines using AS-PaRac1, but it can also

induce shrinkage of potentiated spines using blue light. Indeed, we found that learning enhanced spine potentiation, and AS-PaRac1 successfully labeled

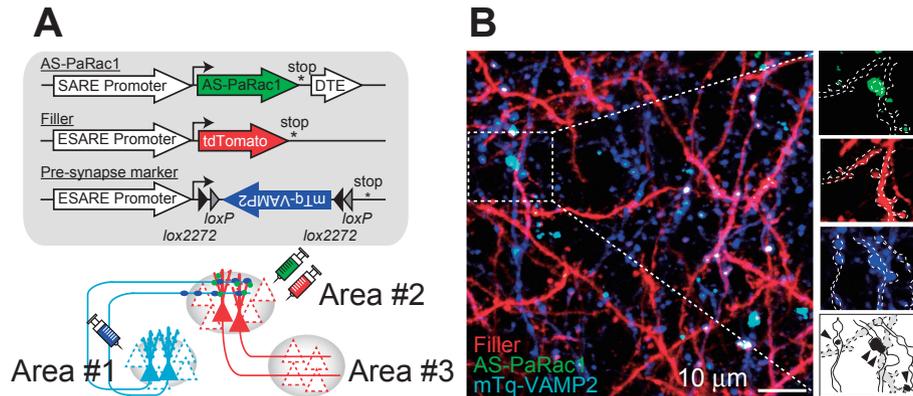


Fig. 6. Multi-scale functional imaging allows simultaneous visualization of both presynaptic and postsynaptic activation. (A) Construct design. For the projection-specific labeling of the presynaptic terminals, CRE-dependent expression of the presynaptic marker is used. (B) Representative image of multi-scale imaging. The merged puncta (triple labelled) are considered Hebbian synaptic plasticity (arrowheads).

70–80% of potentiated spines (Fig. 5C). Furthermore, blue light irradiation induced the shrinkage of the learning-related AS-PaRac1-containing spines (Fig. 5D), which was accompanied by disruption in an acquired skill after learning (Fig. 5E, F). Thus, using this method, we were able to show a direct causal link between synaptic degeneration and learning. The results obtained using this probe provided the first direct evidence that dendritic spine plasticity underlies behavior. Although more work will be required to determine whether this method can be used to understand psychiatric disorders, multiple lines of evidence strongly suggest that the dysregulation of synaptic plasticity underlies a variety of psychiatric disorders, including SZ, autism, depression, and post-traumatic disorder. Thus, visualization and manipulation of possible synaptic pathology using AS-PaRac1 could provide mechanistic insights into synaptic resolution. For instance, there are further potential applications of this technique. Among the many forms of synaptic plasticity, Hebbian plasticity is considered the pivotal mechanism underlying the activity-dependent reorganization of neuronal ensembles. This type of plasticity is induced by correlations between pre- and postsynaptic firing, which result in a positive feedback loop that increases postsynaptic firing rates. Thus, if we could visualize simultaneous presynaptic and postsynaptic activations, it would be possible to monitor Hebbian synaptic plasticity (Fig. 6). For the labelling of the presynapse, we can take advantage of vesicle associated membrane protein 2 (VAMP2), a presynaptic protein important for vesicle docking and fusion, along with a fluorescence protein with a

different color spectrum for use as the filler to visualize postsynaptic neurons. Probes for the pre-synapse (VAMP2-mTurquoise), postsynapse (AS-PaRac1), and postsynaptic neurons are expressed in an activity-dependent manners and can be designed for rapid decay through means of a destabilizing fusion signal. The presence of a double-floxed inverted open reading frame (DIO) and Cre recombinase allow projection pathway-specific visualization and synaptic manipulation (Fig. 6). The uniqueness of this method is that it allows us to detect not only synaptic plasticity (micro imaging) but also the plastic reorganization of neuronal circuits in different brain areas (macro imaging). This multi-scale imaging could help to elucidate the dynamic nature of synaptic potentiation in a specific neuronal circuit in the vast network of neuronal circuits in the brain. In addition, a combination of tissue clearing and light-sheet microscopy will allow researchers to perform a comprehensive analysis of how such potentiation is distributed and altered throughout the brains of psychiatric model mice, even at a single-spine resolution. Once we identify a synaptic ensemble that breaks the balance in a neural circuit, it should be possible to induce disconnection of this synaptic ensemble via photoactivation of AS-PaRac1. Subsequent behavioral changes or the absence thereof could provide evidence about whether or not the synaptic ensemble is a cellular event that contributes to the pathophysiology of the disease. With the advent of this powerful tool, we can now focus on how synaptic connections and plasticity are altered in disease states. Optogenetic findings can infer causality. This is necessary for the establish-

ment of circuit-centric therapeutics, which overcome the limitations of molecular- and chemistry-based drug approaches.

### Conclusion

The review examined the involvement of synaptic disturbances in the pathophysiology of SZ. The essential and minimal components underlying SZ, especially at the neuronal circuitry level, remain unknown. This has prevented the development of curative and radical treatments for this disorder. Indeed, despite the accumulation of evidence for the presence of synaptic dysregulation in SZ, the success of therapeutic strategies designed to modulate glutamatergic synaptic transmission has been limited.<sup>112)</sup> However, state-of-the-art techniques such as optogenetics and *in vivo* two-photon imaging of animal models of SZ will allow us to decipher the causal effects of particular molecular and cellular events. If a candidate disturbance is translatable and testable in humans, this will allow us to identify specific targets and will thus aid the development of therapeutic strategies for the treatment of SZ.

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### Author contribution

A.H.-T. wrote the paper, and K.O.-N. and T.Y. assisted with figure preparation.

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## Profile

Kisho Obi-Nagata was born in Akita prefecture in 1991 and graduated from Gunma University School of Medicine in 2017. In order to be a good physician, he thought critical reasoning and logical thinking would markedly help his clinical skills and joined the basic neuroscience laboratory in spite of being as the freshman. Having performed experiments during his six-year program at medical school, he was fascinated by the significance of science that might ultimately bridge the great gap between what science knows and what physicians do. He had no hesitation in enrolling in the graduate school and decided to enter the Ph.D. program at Gunma University (Lab of Medical Neuroscience, Prof. Akiko Hayashi-Takagi). He is now seeking to reveal the significance of pathological synaptic disturbance in psychiatric model mice.



## Profile

Yusuke Temma was born in Hiroshima prefecture in 1995 and graduated from Kyushu University in 2019. During Bed Side Learning in the Medical School, he saw a lot of patients suffering from chronic diseases such as psychiatric disorders. Moreover, he was surprised that the causes of such disorders are poorly understood and no curative treatments are available. Thus, he decided to become a physician-scientist and started his career as a graduate student in the Hayashi-Takagi laboratory at Gunma University, with the aim of elucidating the development of and mechanism-oriented treatments for psychiatric disorders.



## Profile

Akiko Hayashi-Takagi was born in Gunma prefecture in 1974 and graduated from Gunma University School of Medicine in 1999. She originally hoped to contribute to medicine as a physician (residency training at Gunma University Hospital). During this residency period, she fully recognized the importance of basic scientific approaches for psychiatric diseases, because the pathophysiology of these diseases remains largely unknown, which has hindered the discovery of curative drugs. Therefore, while keeping her ultimate goal to contribute to psychiatry and patients with psychiatric conditions, she entered the Ph.D. program at Gunma University in 2001 (Ph.D., 2005). Through postdoctoral training at RIKEN (2005–2007) and Johns Hopkins University (2007–2010), followed by a junior faculty position at the University of Tokyo (2010–2016), she has been dedicated to research on synapses, the connections between the two neurons, because accumulating evidence suggests that the malfunction of synapses is a crucial contributing factor in psychiatric diseases. To demonstrate this hypothesis, her laboratory (Full Professor at Gunma University, since 2016) is performing a multi-scale analysis (molecule, synapse, cell, circuit, and behavior) of synaptic pathology of animal models of psychiatric disorders as well as the establishment of a novel synaptic optoprobe. Furthermore, to amplify her research vision, she is now organizing a large-scale grant (MEXT, Grant-in-Aid Scientific Research on Innovative areas FY 2018–2022, “Constructive understanding of multi-scale dynamism of neuropsychiatric disorders”) as the project director.

