Mutations in DISC1 alter IP₃R and voltage-gated Ca²⁺ channel functioning, implications for major mental illness

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Disrupted in Schizophrenia 1 (DISC1) participates in a wide variety of developmental processes of central neurons. It also serves critical roles that underlie cognitive functioning in adult central neurons. Here we summarize DISC1’s general properties and discuss its use as a model system for understanding major mental illnesses (MMIs). We then discuss the cellular actions of DISC1 that involve or regulate Ca²⁺ signaling in adult central neurons. In particular, we focus on the tethering role DISC1 plays in transporting RNA particles containing Ca²⁺ channel subunit RNAs, including IP3R1, CACNA1C and CACNA2D1, and in transporting mitochondria into dendritic and axonal processes. We also review DISC1’s role in modulating IP₃R1 activity within mitochondria-associated ER membrane (MAM). Finally, we discuss DISC1-glycogen synthase kinase 3β (GSK3β) signaling that regulates functional expression of voltage-gated Ca²⁺ channels (VGCCs) at central synapses. In each case, DISC1 regulates the movement of molecules that impact Ca²⁺ signaling in neurons.

Introduction

Societies across the globe share a consensus of what constitutes basic, productive human social behavior. This shared reality breaks down in individuals with major psychotic disorders, most notably in schizophrenia (Schz). The inability of most schizophrenics to function as independent adults, due to hallucinations and delusions, cognitive disorganization, and in some cases depressed psychomotor functioning (lack of speech, lack of spontaneous movement and various aspects of blunted emotion), profoundly affects the individual's quality of life and is a burden on their families as well as to society. Despite recognition since early civilization of 'madness', the defining of Schz as a distinct psychosis only occurred in the early 20th century [1]. Even with 100 years of study and treatment, the underlying causes of schizophrenia have been difficult to identify. Epigenetic and epistatic factors, as well as expression of unknown genes that protect against mental illness, obscure the influence of genetic inheritance.

However, this dearth of information is changing. A series of landmark genome-wide association studies (GWAS) have provided new insights into the genetic underpinnings of the disorder. Surprisingly, the psychotic disorders, bipolar disorder (BPD), major depression disorder (MDD), and Schz share sufficient overlapping GWAS hits that more recent bioinformatics studies have pooled thousands of individuals under the general heading of major mental illness (MMI). Identification of susceptibility genes common to BPD, MDD, and Schz is consistent with the observation that these three disorders have overlapping symptoms and treatment strategies. Interrogation of GWAS [2–6], as well as chromatin [7] transcriptome [8,9], exome (the protein coding region of the genome) [10], signaling pathway database analyses [4,11], and recent polygenic analyses [10,12–13], have revealed hundreds of genes with single nucleotide polymorphisms (SNPs) that associate with these three major psychotic disorders.
From interrogating tens of thousands of human genomes, bioinformatics studies have found associations between common variants in genes, where each SNP contributes mild susceptibility for Schz, however in combination with multiple other susceptibility loci, appear sufficient to give rise to Schz. Rare variants have higher penetrance in up to 20% of Schz cases. These mutations nearly always give rise to symptoms and usually are idiopathic in that the parents of the affected individual are healthy. Lastly, a small number of families worldwide have been identified that carry a gene disruption resulting in high penetrance for MMI in multiple generations. Interestingly, the heritability incidence for Schz is estimated to be at least 70% [14–17] and as high as 80% between twins [18], yet our understanding of how heritability functionally contributes to the etiology of MMI is at best superficial. One strategy to gain functional insight is to examine the biology of these identified genes in healthy individuals to begin to understand their role in cognition.

In this review, we highlight the gene, Disrupted in Schizophrenia 1 (DISC1), the earliest gene discovered that associates with MMI disorders. Its discovery marked the beginning of research on the molecular basis of psychiatric disorders. Jacob et al. (1970) identified a large Scottish family in which multiple generations of family members (more than four generations) exhibited high penetrance for MMI [19]. As many as 70% of the carriers exhibit major psychotic symptoms associated with BPD, MDD, and/or Schz [20–26]. These family members carry a balanced translocation from chromosome 1q42.1 to 11q14.3 [23]. The break point, identified in 2000, occurs within the DISC1 gene resulting in expression of a truncated DISC1 protein that can exhibit intergenic splicing with TRAX or translin-associated factor X (TSNAX) [23]. Many DISC1-binding partners have been identified; how its truncation may alter its interactions with these proteins to precipitate MMI is under intense investigation (Figure 1).

In the following sections, we briefly review the strengths of DISC1 as a useful model system for interrogating the cellular basis of MMI. We then summarize DISC1 gene organization, how its expression affects neuron morphology, and its role in moving organelles along microtubules. Lastly, we focus on the role DISC1 plays in regulating calcium (Ca2+) physiology at adult central synapses by examining its control over IP3 receptors, located in mitochondria-associated endoplasmic reticulum (ER) membrane (MAM), and in regulating voltage-gated Ca2+ channel (VGCC) subunit expression at synapses. While DISC1 has been implicated in binding many proteins to affect multiple cellular functions throughout ontogeny, this review is not exhaustive, but rather, attempts to pull together studies examining cellular changes in Ca2+ signaling in human neurons and human induced pluripotent stem cells (iPSCs) as well as in rodent DISC1 model systems. For earlier insights on DISC1 function and on its regulation of Ca2+ signaling through glutamate receptors, we refer the reader to a number of excellent earlier reviews [27–31]. Additionally, while altered Ca2+ signaling may create a broad-based susceptibility to certain MMI, we are aware that alternate causes of MMI may arise from disruption in other basic physiological processes including immune functioning. See recent reviews for insightful discussion of immune contributions to MMI [32,33].

**DISC1 is a useful model to study MMI**

The translocation first revealed in the DISC1 gene is a historic example of a rare genetic event occurring in the Scottish family where the proband did not have Schz. Genetic linkage in this pedigree associates with Schz (LOD = 3.6) as well as a broad phenotype (LOD 7.1) for BPD, MDD, and Schz [23,24]. An ever growing body of data, summarized below, point to a role for DISC1 in normal cognition, and as such, serves as a useful model for probing underlying changes that occur in MMI.

**Karyotype analysis**

Extensive Karyotype analysis of the Scottish family pedigree revealed that family members carry either no alteration, or a combination of three cytogenetic abnormalities observed by Karyotype analysis. These include: (1) a balanced translocation t(1q42.1 to 11q14.3); (2) a large constriction in the q arm below the centromere of chromosome 1 but above the translocation site; and/or (3) a Robertsonian translocation, characterized by a break at the asymmetric centromere of group D chromosomes (chr13–15), where the long arms fuse to form a single long chromosome [19,23,24]. A large research group at the University of Edinburgh has concentrated on tracking members within the family tree carrying the t(1:11) balanced translocation only and found that this genotype tracks with susceptibility to MMIs, specifically BPD, MDD, and Schz, through multiple generations; see Porteous et al., 2014 [34]. These findings support the argument that the translocation alone is sufficient for increased susceptibility to MMI.

**Additional families with mutations in DISC1**

While the possibility that DISC1 confers susceptibility to MMI generated initial excitement, it was not clear that the psychosis associated with the Scottish family was due to (1) nonfunctional DISC1, (2) other genes disrupted by the
Figure 1. Schematics of DISC1 organization and key interaction sites

(A) DISC1 mRNA is composed of 13 exons. Red arrows denote the translocation break site in the Scottish family and the 4-nucleotide deletion from the American family. Blue arrows show RNA locations that give rise to amino acid substitutions, which result in altered nerve cell function and/or behavior. Gray bars identify RNA sequences that give rise to DISC1 self-association and oligomerization. (B) Schematic of full length 854 amino acid DISC1 protein showing its putative globular or disordered head region and a long coiled-coil region. The relative bindings sites are shown for key proteins (blue bars) and mRNA (red bar).

translocation, (3) coprecipitation of multiple disrupted genes, or (4) altered chromatin structure. One way to resolve the importance of the DISC1 truncation was to find other families with altered DISC1 sequence with high penetrance for MMI. Additional families have been identified that only carry a small deletion of nucleotides in the DISC1 gene that results in premature termination, including Finnish [35–40], American [41,42], Chinese [43,44], and Taiwanese [45] families. These findings suggest that the truncated (tr) DISC1, observed in the Scottish family, rather than other genes within or near the disrupted regions of chromosomes 1 and 11, is sufficient for conferring MMI susceptibility.

**DISC1 as a susceptibility gene in genetic studies of MMI**

In early GWAS for MMIs, DISC1 was not identified as a susceptibility gene raising questions about its function and its use as a model system for MMI. Additionally, no other genetic study implicated DISC1 in MMI due to rare exonic variation, rare copy number variation (CNV), or common variations [10,46–48]. Initially, meta-analysis of common DISC1 SNPs also found no evidence of genome-wide association with Schz [49]. However, with increased numbers of samples in databases, recent meta-analyses identified DISC1 polymorphisms and CNV that associate with Schz [50–52]. Moreover, smaller, more focused GWAS have identified DISC1 as a susceptibility gene for MMI, including

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six ultrarare non-synonymous amino acid substitutions in DISC1 [53,54], rare missense mutations found in a Swedish cohort [55], and an excess rare variant in exon 11 [56].

**DISC1 mutations and association with MMI**

A limitation of GWAS and many CNV studies of MMI susceptibility genes is that they do not identify whether any of these variants affect function [6]. However, a number of DISC1 variants that cause cellular and behavioral abnormalities in model systems also associate with Schz [53] and recurrent MDD [57]. Most notably, an ultrarare DISC1 variant R37W in a case of MDD transmitted to two affected offspring [53]. Additionally two SNPs in intron 9 (rs821577 and rs2295959) show female-specific associations with anxiety, depression, and neuroticism in elderly Scottish subjects [58], and female-specific association with Schz in Han Chinese subjects [43], and with Schz in a Japanese population [59]. Most useful for examining the cellular role of DISC1 are several different amino acid substitutions of Leu607, located in exon 9 and conserved across humans, mice, rats, pufferfish, and zebrafish (Figure 1A). One of these, a Leu607Phe substitution correlates with schizoaffective disorder in an American family [42].

**Broad versus specific behavioral phenotypes found with mutant DISC1**

Because of the high penetrance of multiple forms of MMI in affected families carrying trDISC1 and recent GWAS identifying DISC1 as a general versus specific susceptibility gene for a particular illness, concern exists that its interrogation will yield only superficial understanding of the etiology of Schz. However, DISC1 mutations do correlate with various brain endotypes including behavioral differences in anhedonia and frontal-lobe-associated memory; electrophysiological changes observed in auditory-event-related potentials (ERPs); and anatomical differences in cortical thickness, hippocampal gray matter volume and white matter integrity [60,61]; all changes observed in Schz individuals. Unlike family members without the mutation, 100% of the Scottish family members tested, who carried the t(1:11) translocation, exhibit increased latency and/or smaller amplitude P300 responses [24], a quantitative measure of cerebral ERPs associated with attention and memory processes [34]. Different trDisc1 mouse models exhibit similar changes in behavior, brain circuitry, and synaptic transmission as Schz individuals. If DISC1 confers a more generalized versus selective risk to MMI, studying its function should lead to identification of specific signaling pathways that become disrupted in different psychotic diseases. Indeed, DISC1 interacts with a plethora of Schz susceptibility genes, reinforcing the research strategy of understanding DISC1 function to gain insight into the etiology of MMI.

**Gene organization and expression of DISC1**

The DISC1 gene is 414.3 kb with 13 exons located in chromosome 1; it is the only known member of its family. Of note, DISC2 is a non-protein coding IncRNA transcribed from the 3' region of DISC1 and made up of one large exon located antisense to exon 9 of DISC1. Little is known about DISC2's function though it is hypothesized to regulate DISC1 expression [23]. The break point within the DISC1 gene of the Scottish family (Figure 1) occurs within intron 8 resulting in the translocation of exons 9–11 to chromosome 11 as well as the majority of DISC2. Full-length DISC1 is 854 amino acids long with an amino-terminus head domain (amino acids 1–347) that contains nuclear localization signals (Figure 1B). Its C-terminus domain (amino acids 348–854), encoded by exons 3-13, contains coiled coil sequences [30]. DISC1 has binding sites for a large number of proteins, including PDE4B, PCM1, NDE1, IP3R1, and glycogen synthase kinase 3β (GSK3β), which are independently implicated as genetic risk factors for Schz and related MMI [27]. Key SNPs have been found either proximal to regions encoding DISC1 protein interaction domains or are predicted exonic splicing enhancer sites, identifying functionally relevant regions of interest for interrogation [62–64]. At least 50 different transcripts of DISC1 are expressed dynamically over time in various brain regions [44,65,66]. Different cell types in adult cortical brain regions express unique profiles of DISC1 transcripts, which in turn yield, or are predicted to yield, unique DISC1 interactomes for a given cell type over a lifetime with greatest interest in hippocampal and cortical glutamatergic pyramidal neurons and GABA inhibitory interneurons [53,62,65,67,68].

The large number of transcripts may help explain how DISC1 serves so many neuronal functions. Different molecular weight proteins stain positive for DISC1 in Western blot analysis [see 30], suggesting that multiple transcripts of DISC1 express as protein. DISC1 is enriched in the adult prefrontal cortex, hippocampus and striatum, brain areas identified as important in MMI [69]. Within these areas, DISC1 plays major roles in neuronal proliferation [70], cell migration, nucleokinesis [71–73], axonal transport, neurite outgrowth, dendrite arborization, spine morphology, synaptogenesis, synaptic transmission, and synapse maintenance [70], [see 27]); all processes that are disrupted in Schz. Moreover, proteomics and interactome studies have identified proteins that interact with DISC1 protein [70,71]. Despite some concerns about specificity of DISC1 antibodies there seems to be consensus on where DISC1 is expressed and which proteins interact with DISC1. As more reagents and model systems are utilized in future studies,
further comparisons among studies on DISC1 expression and function will occur. Moreover, commercial DISC1 antibodies are now available allowing multiple labs to use the same reagents, facilitating comparison of results gathered from probing DISC1’s functional interactions in different model systems.

Postmortem analysis of human hippocampus and dorsolateral prefrontal cortex revealed that expression of short DISC1 variants is higher in utero than postnatally [66]. Though its levels decrease postpartum, DISC1 expression remains critical in adult brain for proper cytoskeletal function conveying neuronal polarity [74,75], axonal transport [63,75–77] and synaptic function [78–81]. These processes are notable since they participate in both development as well as adult synaptic plasticity. Enrichment of short protein isoforms of DISC1 occurs in Schz brains [66], suggesting that persistence of their elevated levels may disrupt both brain development as well as adult cognition. More research is needed to understand the significance of the widespread distribution of DISC1 splice variants [11,65] [see also [72]] as well as, which isoforms dynamically interact with, and affect the function of various proteins in different subcellular locations over a lifetime [82–84] (Figure 1B). With so many binding sites for proteins with different cellular roles, one can begin to imagine how mutant or trDISC1 could create unique pathologies in different cell types [85].

A notable feature of DISC1 is that in addition to binding many proteins, it is its own binding partner [66]. Co-immunoprecipitation studies show that DISC1 self-associates through a domain proximal to where the t(1,11) break occurs in the Scottish family [86]. trDISC1 functions as a dominant negative, binding to full-length DISC1 to form insoluble aggregates (Figure 1A). These aggregates result in lowered DISC1 expression levels in human iPSCs and in mouse hippocampus, as well as abnormal cognitive function [76,87]. DISC1 aggregates are recruited to aggresomes that also attract soluble DISC1, but not to Golgi, ER or endocytic pathways [76,88,89]. Aggregated DISC1 shows minimal ability to return to the cytosolic fraction. Rather, it is degraded by the autophagy pathway. Insoluble DISC1 has been found in brain tissue of patients suffering from MMI [76,88]. These aggresomes are reminiscent of protein precipitate that accompany cognitive decline in neurodegenerative diseases, such as in Parkinson’s Disease, Alzheimer’s Disease, and frontotemporal dementia [90].

**Known functions of DISC1**

How truncation of DISC1 contributes broad risk for MMI is a key question that has been probed intensively for more than 20 years. Key morphological changes in CNS neurons provide clues as to how loss of functional DISC1 may increase susceptibility to MMI. Decreased numbers of synaptic spines in cortical regions of autopsied Schz brains are accompanied by elongated dendrites with reduced branching [91]. A strikingly similar morphological profile recapitulates in primary cortical neurons from a mouse model that expresses a C-terminally truncated form of Disc1 under the control of a Tet-off system in cortex, striatum and hippocampus. Expression of trDISC1 results in decreased neurite outgrowth in primary cortical neurons and in reduced levels of SNAP-25 in the forebrain area of young mouse pups [92], suggesting decreases in synapse maturation accompany abnormal arborization. These mice also exhibit sex-dependent changes in behavior, including increased locomotor activity and abnormal social behavior in males while females exhibit impaired spatial reference memory, suggesting that these observed cognitive changes reflect the synaptic changes brought about by trDISC1 expression [92]. Additional interrogation of DISC1’s role in cell morphology reveals that loss of its N-terminus primarily disrupts normal nerve cell proliferation and movement [93], while the C-terminus primarily regulates dendrite morphology and synaptic function. These findings fit with observations that DISC1 transcripts, located in adult neuronal processes, are primarily C-terminal containing variants, suggesting that active regulation of dendrite morphology by DISC1 contributes to proper synaptic transmission [65,94–97].

At the subcellular level, DISC1 is considered a developmental hub protein, because it has no enzymatic function of its own, but rather incorporates into scaffolding where it binds multiple proteins to form signaling complexes that influence many stages of neuronal ontogeny both temporally and spatially [98] [for review; [86,99,100]]. In adulthood, DISC1 serves another critical function as an adaptor protein by tethering cargo to a molecular motor: either kinesin-1 motor complex [101,102] or dynein for anterograde and retrograde movement along microtubules, respectively [96,74,98]. Rather than affecting the rate of movement, DISC1 controls what and when cargo moves along microtubules (Figure 2A). Identified cargos include RNA particles, membraneless granules that contain mRNAs and RNA-binding proteins for controlling localized translation [see [103] for review], as well as mitochondria and synaptic vesicles (SVs) [79,81]. At presynaptic nerve endings, DISC1 regulates expression levels of proteins involved in initiating synaptic transmission [78]. Postsynaptically, DISC1 plays a necessary role in synaptic plasticity by regulating protein movement in and out of the postsynaptic density in a similar manner to regulating cargo movement along microtubules [93,104,105]. Thus, DISC1’s role in spines may be better described as a tethering rather than a scaffolding function where it controls protein movement critical for postsynaptic signaling. At synapses, DISC1’s tethering functions also appear critical in controlling Ca²⁺ signaling. In the following sections, we discuss how DISC1 may alter...
Figure 2. In adult central neurons, DISC1 regulates cargo transport along microtubules (striped green) and Ca^{2+} transfer from ER to mitochondria at MAM.

(A) DISC1 serves as a tether for mitochondria, RNA (brown strands), RNA particles (yellow) and SVs filled with transmitter (pale blue). DISC1 binds to a kinesin motor complex to regulate transport of various cargo into dendrites (blue arrows) and/or nerve terminals (purple arrow).

(B) DISC1 binding to IP₃Rs within MAMs lowers the amount of Ca^{2+} transferred via VDAC channels into mitochondria, protecting them from excitotoxicity. DISC1 does not appear to regulate ER Ca^{2+} release by the ryanodine receptor 2 (RYR2).

Ca^{2+} signaling due to its interactions with (1) RNA particles containing Ca^{2+} channel transcripts, (2) IP₃Rs located in mitochondria-associated ER membranes (MAMs), and (3) VGCCs in nerve terminals.
**DISC1 tethers to molecular motors-specific mRNA particles enriched in transcripts involved in Ca^{2+} signaling and membrane excitability**

Proteomic screens for DISC1 interactors identified several RNA-binding proteins, including hematopoietic zinc finger protein (HZF), found in RNA-transport particles [106]. DISC1 has an arginine-rich motif (ARM) region containing a nuclear localization signal (NLS), which may be required for mRNA export from the nucleus. The mRNA of inositol-1,4,5-triphosphate receptor type 1 (Itpr1) itself binds DISC1 at an ARM in its N-terminal region. DISC1 binding to Itpr1 is facilitated by HZF binding to both DISC1 and to a distinct binding site on Itpr1 mRNA. DISC1 interaction with HZF and kinesin-1 is required for transport of Itpr1 mRNA along microtubules [101,107]. These findings fit a model proposed by Tsuboi et al. (2015) [106] where kinesin-1 transports the DISC1–HZF–Itpr1 mRNA complex into distal dendrites of hippocampal neurons similarly to particles formed of mRNAs, RNA-binding proteins, adaptor proteins (such as DISC1) and a molecular motor such as Kinesin-1 [101,107] (Figure 2A).

Evidence in favor of this model is that expression of a dominant-negative mutant of kinesin heavy chain protein KIF5A inhibits transport of Itpr1 mRNA. Similarly, depletion of DISC1 negatively impacts transport of Itpr1 mRNA into dendrites, whereas overexpression of DISC1 enhances its transport [106]. These findings are consistent with DISC1 functioning as a cargo adapter, or tether, linking the bound Itpr1 mRNA to the kinesin-1 complex for transport along microtubules. Whether DISC1 binds to RNA particles containing Itpr1 mRNA, and/or directly to Itpr1 mRNA to transit into neuronal processes requires further investigation. Nevertheless, these interactions have functional significance since Itpr1 mRNA and DISC1 colocalize in hippocampal dendrites where release of Ca^{2+} from IP_{3}R is critical for initiating long-term changes in synaptic plasticity [108–113]. Disc1 knockout mice show normal brain cytoarchitecture [68] but exhibit abnormal synaptic activity, impaired maintenance of LTP, and altered emotional behaviors [114]; all functions that involve synaptic plasticity and cognitive behaviors often disrupted in Schz. This phenotype was also observed in hippocampal slices exposed to a cell permeable peptide, which blocks DISC1 binding to Itpr1 mRNA [106], thus linking DISC1 to Ca^{2+}-mediated synaptic plasticity.

In addition to Itpr1 mRNA, at least several hundred mRNAs, packaged into RNA granules, are transported into dendritic spines [115]. From mouse brain extracts and hippocampal lysates, a subset of these transcripts immunoprecipitate with full-length DISC1 protein when using a C-terminal antibody. These transcripts were at least two-fold enriched in the DISC1 immunoprecipitate compared with an IgG immunoprecipitate. Those identified included the pore-forming subunit of the VGCC CaV1.2 (Cacna1c), and its accessory subunit δ2 (Cacna2d1); K_{c}3.1, a delayed rectifier K^{+} channel (Kcnc1), expressed in fast spiking GABA interneurons; and K_{c}3.4, a second K^{+} channel family member (Kcnc4). The interactions appear direct since in vitro RNA binding assays confirmed GST–hDISC1–N1 interaction with biotin-labeled 3′ UTR mRNA of each gene but not with GST alone or to 3′ UTR of other transcripts such as the sodium channel Na_V2.1 (Scn2a), or to the coding sequence of Cacna2d1 [106]. Taken together, these findings indicate that DISC1 binds to a subset of mRNAs encoding proteins that regulate membrane excitability and Ca^{2+} influx. Decreased functional DISC1 would be predicted to lower transport of mRNA to critical sites, consequently decreasing expression and compromising the normal complement of proteins regulating Ca^{2+}-dependent events.

**DISC1 tethers mitochondria to molecular motors to facilitate movement of mitochondria to areas of high metabolic demand**

DISC1 also tethers mitochondria to molecular motors (Figure 2A), positively affecting their anterograde axonal transport [116–118]. Knockdown of DISC1 in neurons significantly lowers the number of mitochondria moving along microtubules from 36 to 16% [116,117]. Two non-synonymous C-terminus SNPs of DISC1, Leu^{607}Phe and Ser^{704}Cys, correlate with MMI and associate with alterations in brain maturation and synaptic function [72,80,119,120]. Leu^{607} lies at the end of DISC1’s putative leucine zipper domain within a region identified as necessary for binding of important factors (e.g., NUDEL, MIP3T, ATF4, ATF5) for neurodevelopment [101]. Leu^{607} also plays a critical role in adult DISC1 function. Expressing the common variants (Leu^{607}/Ser^{704}) or the Cys^{704} variant in DISC1−/− neurons rescues transport. In contrast, the Leu^{607}Phe mutation, which correlates with schizoaffective disorder in an American family [42], is unable to correctly rescue mitochondrial movement. Interestingly, the rare human N-terminal SNP, R37W, also lowers mitochondrial trafficking [121], suggesting that specific residues in both the N- and C-termini of DISC1 may play significant roles in controlling mitochondrial movement. Indeed, overexpression of DISC1 raises the numbers of motile mitochondria to 42% compared with 27% [116,117]. However, none of the DISC1 variants alter the...
velocity of movement, suggesting DISC1 increases the percent of mitochondria in transport rather than controlling the rate of transport, as mentioned above.

Movement of mitochondria by its molecular motor involves dynamic interactions among the kinesin-1 family motor KIF, the trafficking kinesin protein TRAK1, and the transmembrane mitochondrial Rho GTPase adaptor Miro1, which has two GTPase domains each flanked by a Ca^{2+}-binding EF hand (Figure 2A). By interacting with proteins within this motor complex, DISC1 promotes Ca^{2+}-sensitive anterograde movement of mitochondria [116,122]. A similar motor complex is found in dendrites that relies on DISC1 interaction with Miro2 and TRAK2 for mitochondrial movement [116]. Syntaphilin (SNPH), an additional interaction partner within the modulatory complex, binds directly to microtubules and mitochondria immobilizing them upon exposure to rises in intracellular Ca^{2+} [123]. For anterograde movement to occur, DISC1 appears to bind both Miro1 and SNPH keeping them from interacting with one another [123]. With the rise in intracellular Ca^{2+}, DISC1 dissociates from the complex allowing SNPH and Miro1 to interact with one another to anchor mitochondria in place (Figure 2A). Furthermore, upon sensing increased Ca^{2+} levels, whether in terminals or dendrites, Miro uncouples the KIF motor from the complex, halting movement of mitochondria along microtubules [123]. Interestingly, in SNPH knockdown studies in cultured cortical neurons, not only does mitochondrial movement increase, but these cells exhibit decreased axonal branching as well as impaired Ca^{2+} buffering in nerve terminals, [124]. These findings suggest that the complement of proteins within a molecular motor complex determines what and when cargo moves, or stops, as well as the direction of movement. Dependency on Ca^{2+} to dock mitochondria in place ensures an energy source at regions with metabolic demand. Whether additional proteins act in concert with DISC1 to facilitate loading of mitochondria on to microtubules awaits further interrogation. Taken together, these observations link DISC1’s role in determining morphological characteristics of a neuron's branching pattern to its Ca^{2+}-sensitive role in tethering and facilitating mitochondrial movement to areas of high metabolic demand such as axon arborization and growth cone formation during development and in synapses during neurotransmission.

**DISC1 regulates transfer of Ca^{2+} from ER to mitochondria via MAM**

Amino acids 1–350 localize DISC1 to a specialized membrane patch, called MAM, where membrane from the two organelles contact one another (Figure 2B). MAMs are dynamically enriched for stress-related proteins, lipid metabolism enzymes, autophagosome markers, and ion pores including IP_{3}Rs and voltage-dependent anion channel 1 (VDAC1), a member of an anion channel family located in the mitochondrial outer membrane that passes both ATP and Ca^{2+} [125]. In cortical neurons, DISC1 colocalizes with IP_{3}R1s to MAMs; their depletion by shRNA-Itpr1 results in less endogenous and flag-tagged recombinant DISC1 in the crude MAM fraction without causing significant decreases in overall DISC1 expression. Surprisingly, DISC1 appears to play no role in the actual tethering of ER to mitochondria at MAMs. Rather, DISC1 blunts IP_{3}R1-mediated Ca^{2+} release from ER by binding selectively to the IP_{3} binding domain and modulatory domains 1, 2, & 3 but not to a suppressor domain or transmembrane domains of IP_{3}R1s [123,126]. DISC1 has no influence on the intrinsic capacity for Ca^{2+} uptake by mitochondria or on basal ER Ca^{2+} levels [123,126]. Consistent with a role in blunting ER Ca^{2+} release, knockdown of DISC1 using shRNA-Disc1 inappropriately increases IP_{3}-dependent Ca^{2+} transfer through the MAM to mitochondria. Moreover, cortical neurons cultured from DISC1L1 embryos, a mouse line that harbors an impaired Disc1 locus [127], exhibit significant increases in ER–mitochondrial Ca^{2+} transfer following exposure to IP_{3}, which reverses with hDISC1 overexpression. However, the reverse is not the case; mitochondrial Ca^{2+} signals contribute little to altered ER Ca^{2+} dynamics occurring with DISC1 knockdown [123].

Within the MAM, DISC1 also associates with EXOC1, normally a member of the exocyst complex, which targets vesicles to specific docking sites in the plasma membrane [128]. EXOC1 overexpression increases, while its knockdown decreases, ER DISC1 levels in both hippocampal neurons and when recombinant DISC1 is expressed in HEK293 cells [129]. In contrast, ryanodine receptor-mediated Ca^{2+} release, stimulated by caffeine, remains normal when either DISC1 or EXOC1 levels is decreased, supporting a model where DISC1 and EXOC1 regulate ER Ca^{2+} dynamics selectively via IP_{3}R1s [129]. In support of this model, the exocyst complex is known to interact directly with IP_{3}R3s in neurons to regulate intracellular Ca^{2+} signaling [130]. DISC1 and EXOC1 do not exhibit additive or synergistic effects, but rather function in the same pathway, with DISC1 acting downstream of EXOC1 [129]. This relationship has functional significance since knockdown of either DISC1 or EXOC1 increases Ca^{2+} release via IP_{3}Rs that normally occurs following ATP-induced purinergic G_{q}
PCR signaling while overexpression of both decreases the Ca^{2+} signal in HEK293 cells (Figure 2B).
Similarly in neurons, DISC1 modulates Ca\(^{2+}\) efflux through IP\(_{3}\)R1s following G\(_{q}\)PCR stimulation. DISC1 modulates IP\(_{3}\)R1-mediated Ca\(^{2+}\) transfer from ER to mitochondria following ATP stimulation of hippocampal neurons or bradykinin stimulation of differentiated neuroblastoma CAD cells as well as dopamine stimulation of hippocampal D1/D2 heterodimer receptors [129]. In each case, DISC1 deficiency (shRNA-bradykinin stimulation of differentiated neuroblastoma CAD cells as well as dopamine stimulation of hippocampal

As with IP\(_{3}\)R1s, GWAS and exome studies searching for MMI susceptibility genes identified multiple hits for VGCC subunit genes (Figures 3A-C), including CACNA1C, the pore forming subunit of the L-VGCC Ca\(_{1.2}\) and CACNA1D, the pore forming subunit of a second L-VGCC, Ca\(_{1.3}\). Also important accessory channel subunits were identified including CACNB2, CACNA2D1 [5,6,132–134], CACNG4, CACNG5, CACNG6, and CACNG8 [135–141]. Moreover, a recent, in depth RNAseq analysis of the Der1 mouse model, which expresses reduced levels of Disc1, revealed prominent dysregulation of several Ca\(_{1.1}\) subunits and Ca\(_{1.2}\). Subsequent proteomics and pathway analysis identified Ca\(^{2+}\) signaling as at risk in Der1 and overlapping with genetic risk factors found in Schz GWAS [68].

Particular excitement has surrounded hits on CACNA1C since Ca\(_{1.2}\) channels mediate certain forms of excitation–transcription coupling in hippocampus that are integral to long-term changes in central synaptic transmission [142,143]. As mentioned above, DISC1 transports VGCC subunit mRNAs, including CACNA1C, into processes [106], linking control of VGCC subcellular location to DISC1. Changes in L-VGCC expression, and/or function as well as location, could precipitate a broad vulnerability to MMI [144]. While L-VGCC mRNA movement into processes decreases with mutant DISC1, Park et al. (2016), using Ca\(^{2+}\) imaging methods, found that cortical neurons, deficient in DISC1 or SNPH, exhibited no change in KCl-stimulated Ca\(^{2+}\) influx in cell bodies compared with control neurons [123]. The authors argue that L-VGCCs often concentrate in somal regions of neurons and concluded that channel function is unaffected by DISC1. However, their imaging assay may not be sensitive enough to detect a change in Ca\(^{2+}\) influx through a particular channel type since cell bodies of central neurons express multiple VGCC types (see Figure 3C), with each type contributing a minority of the Ca\(^{2+}\) current. Additionally, all of them inactivate under conditions of sustained KCl-mediated depolarization [145] making it difficult to ascertain whether DISC1 regulates particular VGCCs.

Under normal physiological conditions, Ca\(^{2+}\) influx through VGCCs contributes to global Ca\(^{2+}\) signaling by positively modulating intracellular Ca\(^{2+}\) release and also to local Ca\(^{2+}\) microdomains [146] to initiate transmitter release from presynaptic boutons and to mediate postsynaptic integration in dendrites and spines [147]. In contrast with DISC1 binding to and modulating IP\(_{3}\)R1 activity, currently there is no evidence that DISC1 interacts directly

**DISC1 indirectly regulates VGCC stability by controlling its phosphorylation by GSK3\(\beta\) and subsequent proteosomal degradation**

GWAS data reveal that susceptibility genes often fall within the same signaling pathway and/or interact with one another (interactome). As with IP\(_{3}\)R1s, GWAS and exome studies searching for MMI susceptibility genes identified multiple hits for VGCC subunit genes (Figures 3A-C), including CACNA1C, the pore forming subunit of the L-VGCC Ca\(_{1.2}\) and CACNA1D, the pore forming subunit of a second L-VGCC, Ca\(_{1.3}\). Also important accessory channel subunits were identified including CACNB2, CACNA2D1 [5,6,132–134], CACNG4, CACNG5, CACNG6, and CACNG8 [135–141]. Moreover, a recent, in depth RNAseq analysis of the Der1 mouse model, which expresses reduced levels of Disc1, revealed prominent dysregulation of several Ca\(_{1.1}\) subunits and Ca\(_{1.2}\). Subsequent proteomics and pathway analysis identified Ca\(^{2+}\) signaling as at risk in Der1 and overlapping with genetic risk factors found in Schz GWAS [68].

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Figure 3. VGCCs are composed of a pore-forming subunit and associated accessory subunits
(A) Linear topology schematic of pore forming CaV\(\alpha_1\) subunits. Domains I–IV contain six homologous transmembrane segments where the S1–S4 segments form a voltage-sensor paddle with multiple positively charged amino acids (+) found in S4. S5–S6 form the pore with the pore loop (P) folding back into the membrane to form the selectivity filter. (B) Schematic of a VGCC complex with CaV\(\alpha_1\) bound to its CaV\(\beta\) and CaV\(\alpha_2-\delta\) subunits (GPI, glycosylphosphatidylinositol anchor). Domain IV and the C-terminal tail have been removed. (C) Evolutionary tree of CaV\(\alpha_1\) subunits.
Figure 4. Loss of DISC1 results in uncoupling of VGCCs from neuronal secretion machinery

(A) DISC1 can bind and phosphorylate GSK3β to tonically inhibit its activity, whereas truncated (tr) DISC1 is unable to phosphorylate GSK3β, releasing it from inhibition. (B,C) SV proteins interact with terminal membrane proteins to form SNARE complexes (see E) that dock vesicles to release sites. Upon Ca²⁺ influx through VGCCs (C), docked vesicles fuse with the plasma membrane to release neurotransmitter (D). (E) trDISC1 releases GSK3β from inhibition allowing it to phosphorylate (maroon spheres) VGCCs leading to their dissociation from the secretion machinery. Once uncoupled, VGCCs are ubiquitinated and degraded by the proteasome.

GSK3β-mediated regulation of Cav1.2 levels to occur in neurons. Rather than searching for a DISC1–GSK3β–Cav1.2 connection in presynaptic terminals or cell bodies, probing for DISC1 regulation of Cav1.2 in postsynaptic spines and dendrites may be more relevant. Whereas Cav2 family members mediate transmitter release at most central synapses, Cav1.2 is highly expressed postsynaptically in dendrites of central neurons where it plays roles in synaptic plasticity and excitation–transcription coupling [150].

**DISC1 regulates Ca²⁺-dependent SV fusion and transmitter release by controlling GSK3β activity**

GSK3β is a negatively regulated kinase where tonic phosphorylation of Ser⁹ suppresses its activity [151]. Dephosphorylation of Ser⁹ relieves GSK3β from tonic inhibition allowing it to autophosphorylate Tyr²¹⁶, which activates its enzymatic activity. Activated GSK3β in turn acts on a wide variety of proteins in addition to L-VGCCs, including proteins involved in SV fusion and transmitter release (Figure 4). Mao et al (2009) investigated what proteins...
are downstream from DISC1 that disrupt transmitter release and synaptic plasticity in adult hippocampus and found DISC1’s N-terminus (residues 211–225) binds GSK3β directly to impede phosphorylation of Ty216 [70]. GSK3β also binds DISC1 in its α-helical coiled-coil-terminal region (residues 356–595). Point mutations in either of these two regions disrupt DISC1 binding to GSK3β. Notable examples include N-terminus DISC1 mutants Q31L and L100P in mice and R264Q in humans (Figure 1B) exhibit decreased binding to GSK3β [152,153]. A C-terminal D453G mutation in mice does not alter DISC1 expression levels but does disrupt its binding to GSK3β, decreasing it more than 50% in whole brain homogenates [154]. The consequences of sustained GSK3β activation in nerve processes include suppression of glutamate release, loss of LTP in hippocampal neurons, and altered behaviors associated with cognitive function [70,148,155].

One obvious place to look for downstream targets mediating DISC1–GSK3β regulation of adult mouse behavior is at central synapses. Zhu et al. (2010) examined whether, and if so, how GSK3β might affect excitation–secretion coupling using the fluorescent protein FM4-64. FM4-64 is taken up by SV where it fluoresces in this acidic environment but is quenched upon release into the neutral extracellular space [148]. They found that recombinant EGFP-tagged (wt)GSK3β inhibits presynaptic vesicle exocytosis, measured from dissociated hippocampal neurons during Hi K+ stimulation. When GSK3β is activated indirectly by wortmannin, wtGSK3β proteins also exhibited decreased whole-cell P/Q (Cav2.1) current compared with neurons transfected with dnGSK3β. Concomitantly, the magnitude of intracellular Ca2+ signals, measured with Fluo3AM, coincided with the changes in P/Q current amplitude. VGCCs are composed of four repeating domains connected by intracellular loops (Figure 3A). Previously protein kinase C (PKC) and calmodulin-dependent protein kinase II (CaMKII) were shown to phosphorylate serine residues within a region of the II–III linker, called the synprint region of N-(Cav2.2) as well as P/Q channels [156]-[157]. The synprint region interacts with SV fusion machinery [for review see [158,159]]. Thus, GSK3β may not only decrease Cav2.1 current, but also disrupt excitation–secretion coupling through phosphorylation of the II–III linker (Figures3A-B).

Zhu et al. (2010) probed this possibility by performing immunoprecipitation studies with synaptosomes to test whether GSK3β disrupts synaptobrevin association with syntaxin and SNAP-25 [148]. These three proteins form the SNARE complex, that mediates vesicle fusion with the terminal membrane (Figures 4A-E). Stimulation of GSK3β by wortmannin decreased while the GSK3β inhibitor SB216763 enhanced their co-immunoprecipitation. Moreover, phosphorylation of tyrosine residues within the P/Q channel II–III linker by recombinant GSK3β inhibited channel association with the three SNARE proteins. Using FRET analysis, the authors showed that GSK3β activation also decreased synaptobrevin dissociation from the SV protein synaptophysin I. Each of these interactions is required for a SNARE complex to mediate efficient exocytosis. Unfortunately, the authors did not test whether GSK3β also might phosphorylate SNARE proteins directly. Nevertheless, the data strongly support a model where phosphorylation of GSK3β may not only decrease Cav2.1 current, but also disrupt excitation–secretion coupling through phosphorylation of the II–III linker (Figures3A-B).

The importance of GSK3β activity in regulating P/Q-VGCC expression is not unique to hippocampal neurons; it also regulates their expression in NPY-expressing arcuate nucleus (ARC-NPY) neurons of the hypothalamus [160]. Western blot findings demonstrated that in ARC-NPY neurons, decreasing extracellular glucose from 10 to 1 mM significantly increases AMP-activated kinase α2 (AMPKα2) phosphorylation within 2 min, acutely activating it. Concomitantly, lowered [glucose] or exposure to an AMPKα2 agonist increases phosphorylation of GSK3β, inhibiting its activity. Chen et al. (2012) also found lowered extracellular [glucose] stimulates an approximate 25% rise in [Ca2+]i, and increases P/Q current selectively by ~40%, while inhibition of AMPKα2 decreases VGCC currents of dissociated ARC-NPY neurons [160]. Consistent with GSK3β phosphorylation by AMPKα2 mediating rises in [Ca2+]i, exposure to LiCl2 during a drop in [glucose] enhanced increases in [Ca2+]i. AMPKα2’s actions are physiologically relevant since the ARC-NPY neurons play a central role in regulating food intake and glucose homeostasis [161,162]. Moreover, recent kinase pathway analysis of iPSC-derived glutamatergic neurons from a patient with a 4-bp mutation in DISC1 found that DISC1 mutant cells had significantly lower AMPKα2 levels compared with neurons derived from a wildtype sibling. These findings reveal a possible role for DISC1 in regulating AMPKα2 [11]. Whether DISC1 also plays a role in regulating P/Q currents in ARC-NPY nucleus neurons has not been examined. Nevertheless, the findings in ARC-NPY and hippocampal neurons suggest a convergent common pathway of action where GSK3β inhibition by AMPKα2 and/or DISC1 relieves P/Q-VGCCs from tonic inhibition.

While Zhu et al. (2010) found active GSK3β decreases P/Q current, SNARE protein association with channels and with each other, as well as SV fusion, they did not probe whether DISC1 plays a role in regulating exocytosis [148]. However, using RNAi technology and a DISC1 knockout mouse model, Tang et al. (2016) interrogated DISC1’s role in activity-dependent neurotransmitter release [78]. SV fusion was imaged by electrophoresing dissociated hippocampal neurons (14–16 DIV) with the synaptic tracer vGpH, a mutant pH-sensitive GFP (pHluorin) [163], fused to the
SV glutamate transporter, vGlut1 [78]. At rest, pHluorin faces the acidic lumen of SVs. However, after SV fusion, vGpH undergoes a ∼20-fold increase in fluorescence intensity in response to the neutral pH of the extracellular solution [164]. Following glutamate exocytosis and vGpH re-uptake, SVs rapidly re-acidify and consequently vGpH fluorescence is quenched. The pH-sensitive properties of vGpH make it a valuable tool for monitoring SV lifecycle at single synapses.

By measuring changes in fluorescence during field stimulation, Tang et al. (2016) imaged hundreds of individual synaptic boutons from cultured rat hippocampal neurons transfected with shRNA targeting Disc1’s exon 2 or 9 [78]. With either shRNA construct, neurons exhibited a slower rise time and lower intensity of vGpH signal during both an initial and second 10 Hz/300 action potential (AP) stimulation period relative to neurons expressing an scr-shRNA. If stimulation duration was increased to 1200 APs, the vGpH response reached the same maximal level for scr- and shRNA-treated neurons, suggesting DISC1 knockdown did not affect the total releasable pool of SVs or the rate of membrane recovery (a measure of endocytosis), but did slow the kinetics of SV fusion. Cultured hippocampal neurons from a mouse model with exons 2 and 3 deleted [114] lack full-length DISC1, the major isoform in mouse brain. These neurons show similar SV cycling defects where the rates and amplitudes of vGpH signals were reduced compared to wildtype neurons. Together these findings indicate that loss of full length DISC1 disrupts rapid exocytosis of SVs from glutamatergic neurons with no obvious effect on the total releasable pool. For these experiments, Tang et al. (2016) examined whether AP-evoked intracellular Ca\(^{2+}\) signals changed with decreased DISC1 [78]. The authors used the SV-targeted Ca\(^{2+}\) sensor SyGCamp3 [165] to detect changes in Ca\(^{2+}\) levels during a 10 Hz train of 300 APs and found a blunted Ca\(^{2+}\) signal in the absence of full length DISC1 expression. Elevating extracellular Ca\(^{2+}\) from 2 to 4 mM Ca\(^{2+}\) increased AP induced Ca\(^{2+}\) signals and restored the vGpH response suggesting a possible action of DISC1 on VGCCs.

In central synapses Ca\(_V\)2.1 (P/Q-VGCCs) and Ca\(_V\)2.2 (N-VGCCs) largely control excitation-secretion coupling [145, 158]. Tang et al. (2016) determined that with their AP stimulation protocol, blocking Ca\(_V\)2.2 with \(\omega\)-conotoxin decreased vGpH signals ∼73% while inhibiting Ca\(_V\)2.1 with \(\omega\)-agatoxin TK decreased the signal by 42% in cultured hippocampal neurons [78]. Perhaps not surprisingly, blocking Ca\(_V\)2.2 occluded the previously observed decrease in exocytosis, while comparing control to DISC1 knockdown neurons. These findings suggest that under these stimulation conditions, DISC1 regulates both Ca\(_V\)2.1 and Ca\(_V\)2.2 mediated SV release from hippocampal neurons with Ca\(_V\)2.2 activity largely responsible for excitation-secretion coupling. Surprisingly, no difference in the fraction of boutons containing Ca\(_V\)2.2 or in the intensity of Ca\(_V\)2.2 labeling in presynaptic boutons were found. The same held true for Ca\(_V\)2.1. Despite the super resolution images, the detailed images appear insufficient to rule out the possibility that DISC1 regulates the number of functional Ca\(_V\)2 channels expressed in membrane associated with active zones.

Therefore, to test for this possibility, Tang et al. (2016) examined the effect of DISC1 on Ca\(_V\)2.2 currents using whole-cell patch clamp methods [78]. They first cotransfected DISC1 with Ca\(_V\)2.2 and axillary subunits into HEK293 cells and found that the presence of DISC1 significantly increased both peak and tail current density with no change in voltage-dependence of activation nor any obvious change in opening kinetics. Recordings of Ca\(_V\)2.1 in cells expressing DISC1 exhibited a similar voltage-independent potentiation of the whole-cell current. These findings are consistent with DISC1 promoting cell surface expression of functional Ca\(_V\)2.1/2.2 VGCCs at least in HEK293 cells with no change in channel gating. It would be useful to determine whether similar changes in whole-cell currents occur in hippocampal neurons as well since the fluorescent images appear to show no change in the percent of synaptic boutons expressing Ca\(_V\)2 VGCCs. Despite the number of Ca\(_V\)2-positive boutons remaining unchanged as well as the intensity of Ca\(_V\)2.2 staining in nerve terminals, their whole-cell recordings from HEK293 cells suggest a significant decrease in functional channels involved in transmitter exocytosis. These seemingly conflicting findings may result simply from insufficient resolution for observing a change in staining intensity in the plasmalemma of the hippocampal boutons. Resolving the apparent difference in findings will provide a better understanding of the mechanism by which DISC1 increases VGCC activity and thus neurotransmitter release. Despite this shortcoming, Tang et al. (2016) is the first report to document VGCC regulation by DISC1 in neurons [78]. Moreover, their findings show that the actions of DISC1 overexpression on VGCCs parallel those of decreasing GSK3β activity in hippocampal neurons [148, 155], and ARC neurons [160]. Whether they act in concert within an active zone signaling microdomain awaits further interrogation.
Concluding remarks and remaining unanswered questions for understanding the cellular basis of mental illness using the DISC1 model

Since the initial description of a Scottish family with high penetrance for MMI and subsequent identification of a translocation defect t(1:11) in affected individuals, a tremendous amount of information is now known about DISC1’s actions at the cellular level and its requirement for normal cognitive functioning. DISC1’s influence on psychiatric illness appears quite broad since it interacts with so many proteins. This review has focused on DISC1’s interactions with proteins that affect Ca^{2+} signaling. The need to fully understand DISC1’s function on cognition will continue to drive research to confirm DISC1 protein expression, understand the functional importance of these different DISC1 splice variants, identify DISC1’s many binding partners, and then interrogate them to better understand DISC1’s regulatory actions on each protein in different types of neurons over time. A common functional theme in adult neurons is that DISC1 regulates movement at the molecular level. Specifically, DISC1 acts as a gate keeper for the movement of a variety of molecules and organelles: mRNA, RNA particles, SV, and mitochondria, by tethering molecules and organelles to molecular motors. Additionally, by dynamically regulating Ca^{2+} flux through IP_{3}R1 at MAMs, critical for proper mitochondrial functioning, DISC1 may control local energy production required for neurite branching, elongation, and synaptogenesis. This realization suggests that one pathology with trDISC1 may be due to excessive Ca^{2+} influx into mitochondria, compromising coupling between its electrical potential and ATP production. DISC1 regulates additional Ca^{2+} physiology by controlling the movement of VGCC subunit mRNA into dendrites and axons, which is predicted to affect their location and expression levels over time and consequently alter excitation-translation coupling occurring at synapses. At adult synapses, DISC1 increases, while GSK3β decreases, VGCC expression to reciprocally tune neurotransmitter release. Whether DISC1, GSK3β, AMPKα, and other known binding partners exist together in a microdomain with VGCCs at or near SV fusion sites is a critical question that remains to be answered.

Indeed, many questions remain about the exact relationship between DISC1 and VGCCs. We are especially interested in knowing whether DISC1 directly interacts with particular VGCC subunits at synapses. Additionally, careful characterization of where and when different splice variants of DISC1 are expressed should allow the field to answer the question of whether loss of one function, or all of them, contribute to MMI susceptibility with expression of trDISC1. Moreover, it is possible that altered splice variant expression ratios in certain cell types during development and into adulthood increase susceptibility to MMI, independently of DISC1 truncation. This could arise from disruption in the normal processes mediating gene splicing rather than a change in DISC1 sequence. Similarly, various combinations of SNPs in DISC1 may be required to elevate MMI susceptibility [85]. These broad questions will take a great deal of interrogation before arriving at answers. However, the increase in DISC1 mouse models, commercially available antibodies, and a host of new nanotechnologies for studying molecules in single central neurons should allow the field to probe further how trDISC1 alters Ca^{2+} signaling via Ca^{2+} channels resulting in increased susceptibility for developing MMI. Current cell and molecular studies, summarized in this review, highlight a new appreciation of a DISC1-Ca^{2+} signaling node as critical for adult cognition.

Data Availability
There are no primary data included in the manuscript as it is a review.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

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25 Muir, W.J., Pickard, B.S. and Blackwood, D.H. (2008) Disrupted-in-Schizophrenia-1. Curr. Psychiatry Rep. 10, 140–147, https://doi.org/10.1007/s11920-008-0025-2
26 Thomson, P.A., Malavasi, E.L., Grunewald, E., Soares, D.C., Borkowska, M. and Millar, J.K. (2013) DISC1 genetics, biology and psychiatric illness. Front. Biol. (Beijing) 8, 1–31, https://doi.org/10.1007/s11515-012-1254-7
27 Bradshaw, N.J. and Porteous, D.J. (2012) DISC1-binding proteins in neural development, signalling and schizophrenia. Neuropharmacology 62, 1230–1241, https://doi.org/10.1016/j.neuropharm.2010.12.027
28 Devine, M.J., Norkett, R. and Kittler, J.T. (2016) DISC1 is a coordinator of intracellular trafficking to shape neuronal development and connectivity. J. Physiol. 594, 5459–5469, https://doi.org/10.1113/jp272187
29 Porteous, D.J., Millar, J.K., Brandon, N.J. and Sawa, A. (2011) DISC1 at 10: connecting psychiatric genetics and neuroscience. Trends Mol. Med. 17, 699–706, https://doi.org/10.1016/j.trendsrenal.2011.09.002
30 Soares, D.C., Carlyle, B.C., Bradshaw, N.J. and Porteous, D.J. (2011) DISC1: structure, function, and therapeutic potential for major mental illness. ACS Chem. Neurosci. 2, 609–632, https://doi.org/10.1021/cn200062k
31 Tropea, D., Hardingham, N., Millar, K. and Fox, K. (2018) Mechanisms underlying the role of DISC1 in synaptic plasticity. J. Physiol. 596, 2747–2771, https://doi.org/10.1113/JP274330
32 Pouget, J.S., Han, B., Wu, Y., Mignot, E., Ollila, H.M., Schizophrenia Working Group of the Psychiatric Genomics C et al. (2019) Cross-disorder analysis of schizophrenia and 19 immune-mediated diseases identifies shared genetic risk. Hum. Mol. Genet. 28, 3498–3513, https://doi.org/10.1093/hmg/ddz2145
33 Comer, A.L., Carrier, M., Tremblay, M.E. and Cruz-Martin, A. (2020) The inflamed brain in schizophrenia: the convergence of genetic and environmental risk factors that lead to uncontrolled neuroinflammation. Front. Cell. Neurosci. 14, 274, https://doi.org/10.3389/fncel.2020.00274
34 Porteous, D.J., Thomson, P.A., Millar, J.K., Evans, K.L., Hennah, W., Soares, D.C. et al. (2014) DISC1 as a genetic risk factor for schizophrenia and related major mental illness: response to Sullivan. Mol. Psychiatry 19, 141–143, https://doi.org/10.1038/mp.2013.160
35 Hennah, W., Tomppo, L., Hiekkalinna, T., Palo, D.M., Kilipinen, H., Ekelund, J. et al. (2007) Families with the risk allele of DISC1 reveal a link between schizophrenia and another component of the same molecular pathway, NDE1. Hum. Mol. Genet. 16, 453–462, https://doi.org/10.1093/hmg/ddi462
36 Hennah, W., Tuulio-Henriksson, A., Paunio, T., Ekelund, J., Varilo, T., Partonen, T. et al. (2005) A haplotype within the DISC1 gene is associated with schizophrenia and other psychiatric disorders. Mol. Psychiatry 10, 1097–1103, https://doi.org/10.1038/sj.mp.4001731
37 Hennah, W., Varilo, T., Keitila, M., Paunio, T., Arjavri, R., Haukk, J. et al. (2003) Haplotype transmission analysis provides evidence of association for DISC1 to schizophrenia and suggests sex-dependent effects. Hum. Mol. Genet. 12, 3151–3159, https://doi.org/10.1093/hmg/ddg341
38 Ekelund, J., Hennah, W., Hiekkalinna, T., Parker, A., Meyer, J., Lonnqvist, J. et al. (2004) Replication of 1q42 linkage in Finnish schizophrenia pedigrees. Mol. Psychiatry 9, 1037–1041, https://doi.org/10.1038/sj.mp.4001536
39 Ekelund, J., Hovatta, I., Parker, A., Paunio, T., Varilo, T., Martin, R. et al. (2001) Chromosome 1 loci in Finnish schizophrenia families. Hum. Mol. Genet. 10, 1611–1617, https://doi.org/10.1093/hmg/10.15.1611
40 Tienari, P., Wynne, L.C., Moring, J., Lahiri, I., Naarala, M., Sorri, A. et al. (1994) The Finnish adoptive family study of schizophrenia. Implications for family research. Br. J. Psychiatry Suppl. 23, 20–26, https://doi.org/10.1192/s00071250000292696
41 Sachs, N.A., Sawa, A., Holmes, S.E., Ross, C.A., DeLisi, L.E. and Margolis, R.L. (2005) A frameshift mutation in Disrupted in Schizophrenia 1 in an American family with schizophrenia and schizoaffective disorder. Mol. Psychiatry 10, 758–764, https://doi.org/10.1038/sj.mp.4001667
42 Hodgkinson, C.A., Goldman, D., Jaeger, J., Persaud, S., Kane, J.M., Lipsky, R.H. et al. (2004) Disrupted in schizophrenia 1 (DISC1): association with schizophrenia, schizoaffective disorder, and bipolar disorder. Am. J. Hum. Genet. 75, 862–872, https://doi.org/10.1086/425586
43 Chen, Q.Y., Chen, Q., Feng, G.Y., Lindpaintner, K., Wang, L.J., Chen, Z.X. et al. (2007) Control association study of Disrupted-in-Schizophrenia-1 (DISC1) gene and schizophrenia in the Chinese population. J. Psychiatr. Res. 41, 428–434, https://doi.org/10.1016/j.jpsychires.2006.01.001
44 Wang, H.Y., Liu, Y., Yan, J.W., Hu, X.L., Zhu, D.M., Xu, X.T. et al. (2018) Gene polymorphisms of DISC1 is associated with schizophrenia: evidence from a meta-analysis. Prog. Neuropsychopharmacol. Biol. Psychiatry 81, 64–73, https://doi.org/10.1016/j.pnpbp.2017.10.008
45 Hwu, H.G., Liu, C.M., Fann, C.S., Ou-Yang, W.C. and Lee, S.F. (2003) Linkage of schizophrenia with chromosome 1q loci in Taiwanese families. Mol. Psychiatry 8, 445–452, https://doi.org/10.1038/sj.mp.4001235
46 Fromer, M., Pocklington, A.J., Kavanagh, D.H., Williams, H.J., Dwyer, S., Gormley, P. et al. (2014) De novo mutations in schizophrenia implicate synaptic networks. Nature 506, 179–184, https://doi.org/10.1038/nature12929
47 Levinson, D.F., Duan, J., Oh, S., Wang, K., Sanders, A.R., Shi, J. et al. (2011) Copy number variants in schizophrenia: confirmation of five previous findings and new evidence for 3q29 microdeletions and 1p13.3 duplications. Am. J. Psychiatry 168, 302–316, https://doi.org/10.1176/appi.ajp.2010.10060876
48 Sztatkiewicz, J.P., O’Dushlane, C., Chen, G., Chambert, K., Moran, J., Neale, B.M. et al. (2014) Copy number variation in schizophrenia in Sweden. Mol. Psychiatry 19, 762–773, https://doi.org/10.1038/mp.2014.40
49 Mathieson, I., Munafò, M.R. and Flint, J. (2012) Meta-analysis indicates that common variants at the DISC1 locus are not associated with schizophrenia. Mol. Psychiatry 17, 634–641, https://doi.org/10.1038/mp.2011.41
50 Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T. et al. (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536, 285–291, https://doi.org/10.1038/nature19057
51 Xu, F.L., Wu, X., Zhang, J.J., Wang, B.J. and Yao, J. (2018) A meta-analysis of data associating DRD4 gene polymorphisms with schizophrenia. Neuropsychiatr. Dis. Treat. 14, 153–164, https://doi.org/10.2147/NDT.S156479
52 Xu, Y., Ren, J. and Ye, H. (2018) Association between variations in the disrupted in schizophrenia 1 gene and schizophrenia: a meta-analysis. Gene 651, 94–99, https://doi.org/10.1016/j.gene.2018.01.069
53 Song, W., Li, W., Feng, J., Heston, L.L., Scarringe, W.A. and Sommer, S.S. (2008) Identification of high risk DISC1 structural variants with a 2% attributable risk for schizophrenia. Biochem. Biophys. Res. Commun. 367, 700–706, https://doi.org/10.1016/j.bbrc.2007.12.117
54 Song, W., Li, W., Noltner, K., Yan, J., Green, E., Grozeva, D. et al. (2010) Identification of high risk DISC1 protein structural variants in patients with bipolar spectrum disorder. *Neurol. Lett.* **486**, 136–140, https://doi.org/10.1016/j.neulet.2010.09.027

55 Moens, L.N., De Rijk, P., Reumers, J., Van den Bossche, M.J., Glasser, W., De Zutter, S. et al. (2011) Sequencing of DISC1 pathway genes reveals increased burden of rare missense variants in schizophrenia patients from a northern Swedish population. *PLoS ONE* **6**, e23450, https://doi.org/10.1371/journal.pone.0023450

56 Green, E.K., Grozeva, D., Sims, R., Raybould, R., Forty, L., Forty, R., Smith, B.D., Morris, A. et al. (2011) DISC1 exon 11 rare variants found more commonly in schizoaffective spectrum cases than controls. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **156B**, 490–492, https://doi.org/10.1002/ajmg.b.31187

57 Thomson, P.A., MacIntyre, D.J., Hamilton, G., Dominiczak, A., Smith, B.H., Morris, A. et al. (2013) Association of DISC1 variants with age of onset in a population-based sample of recurrent major depression. *Mol. Psychiatry* **18**, 745–747, https://doi.org/10.1038/mp.2012.117

58 Harris, S.E., Hennah, W., Thomson, P.A., Luciano, M., Starr, J.M., Porteous, D.J. et al. (2010) Variation in DISC1 is associated with anxiety, depression and emotional stability in elderly women. *Mol. Psychiatry* **15**, 232–234, https://doi.org/10.1038/mp.2009.88

59 Hashimoto, R., Numakawa, T., Ohtishi, T., Kumamaru, E., Yagasaki, Y., Ishimoto, T. et al. (2006) Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. *Hum. Mol. Genet.* **15**, 3024–3033, https://doi.org/10.1093/hmg/ddc244

60 Callicott, J.H., Straub, R.E., Pezawas, L., Egan, M.F., Mattay, V.S., Hariri, A.R. et al. (2005) Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 8627–8632, https://doi.org/10.1073/pnas.0500515102

61 Cannon, T.D., Hennah, W., van Erp, T.G., Thompson, P.M., Lonnqvist, J., Huttunen, M. et al. (2005) Association of DISC1/TRAX haplotypes with schizophrenia, reduced prefrontal gray matter, and impaired short- and long-term memory. *Arch. Gen. Psychiatry* **62**, 1205–1213, https://doi.org/10.1001/archpsyc.62.11.1205

62 Liu, C.M., Liu, Y.L., Huw, H.G., Fann, C.S., Yang, U.C., Hsu, P.C. et al. (2019) Genetic associations and expression of extra-short isoforms of disrupted-in-schizophrenia 1 in a neuropsychiatric subgroup of schizophrenia. *J. Hum. Genet.* **64**, 653–663, https://doi.org/10.1038/s41365-019-0597-1

63 Nicodemus, K.K., Callicott, J.H., Higier, R.G., Luna, A., Nixon, D.C., Lipska, B.K. et al. (2010) Evidence of statistical epistasis between DISC1, CIT and NDE11 impacting risk for schizophrenia: biological validation with functional neuroimaging. *Hum. Genet.* **127**, 441–452, https://doi.org/10.1007/s00439-009-0782-y

64 Liu, Y.L., Fann, C.S., Liu, C.M., Chen, W.J., Wu, J.Y., Hung, S.I. et al. (2006) A single nucleotide polymorphism fine mapping study of chromosome 1q42.1 reveals the vulnerability genes for schizophrenia, GNPAT and DISC1: Association with impairment of sustained attention. *Brain* **129**, 554–562, https://doi.org/10.1093/biobpsy/2006.04.024

65 Nakata, K., Lipska, B.K., Hyde, T.M., Ye, T., Newburn, E.N., Morita, Y. et al. (2009) DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 15873–15878, https://doi.org/10.1073/pnas.0903413106

66 Newburn, E.N., Hyde, T.M., Ye, T., Morita, Y., Weinberger, D.R., Kleinman, J.E. et al. (2011) Interactions of human truncated DISC1 proteins: implications for schizophrenia. *Transl. Psychiatry* **1**, e30, https://doi.org/10.1038/tp.2011.31

67 Wilkinson, B., Evgurov, O.V., Zheng, D., Hartel, N., Knowles, J.A., Graham, N.A. et al. (2019) Endogenous cell type-specific disrupted in schizophrenia 1 interactomes reveal protein networks associated with neurodevelopmental disorders. *Biol. Psychiatry* **85**, 305–316, https://doi.org/10.1016/j.biopsych.2018.05.009

68 Bonneau, M., Sullivan, S.T.O., Gonzalez-Lozano, M.A., Baxter, P., Gautier, P., Marchisella, E. et al. (2021) Functional brain defects in a mouse model of a chromosomal t(1;11) translocation that disrupts DISC1 and confers increased risk of psychiatric illness. *Transl. Psychiatry* **11**, 135, https://doi.org/10.1038/s41398-021-02156-3

69 Kirkpatrick, B., Xu, L., Cascella, N., Otsu, T., Sawa, A. and Roberts, R.C. (2006) DISC1 immunoreactivity at the light and ultrastructural level in the human neocortex. *J. Comp. Neurol.* **497**, 436–450, https://doi.org/10.1002/cne.21007

70 Mao, Y., Ye, X., Frank, C.L., Madison, J.M., Koehler, A.N., Doud, M.K. et al. (2009) Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. *Cell* **136**, 1017–1031, https://doi.org/10.1016/j.cell.2008.12.044

71 Brandon, N.J. and Sawa, A. (2011) Linking neurodevelopmental and synaptic theories of mental illness through DISC1. *Arch. Gen. Psychiatry* **68**, 490–492, https://doi.org/10.1002/ajmg.b.31187

72 Chubb, J.E., Bradshaw, N.J., Soares, B.C., Porteous, D.J. and Millar, J.K. (2008) The DISC locus in psychiatric illness. *Mol. Psychiatry* **13**, 36–64, https://doi.org/10.1038/sj.mp.4002106

73 Wynshaw-Boris, A. (2009) Elongator bridges tubulin acetylation and neuronal migration. *Cell* **136**, 393–394, https://doi.org/10.1016/j.cell.2009.01.024

74 Morris, J.A., Kandpal, G., Ma, L. and Austlin, C.P. (2003) DISC1 (Disrupted-in-Schizophrenia 1) is a centromere-associated protein that interacts with MAP1A, MPPT3, ATF4/5 and NUDEL: regulation and loss of interaction with mutation. *Hum. Mol. Genet.* **12**, 1591–1608, https://doi.org/10.1093/hmg/ddg162

75 Brandon, N.J., Handford, E.J., Schurov, I., Rain, J.C., Pelling, M., Duran-Jimenez, B. et al. (2004) Disrupted in schizophrenia 1 and Nudel form a neurodevelopmentally regulated protein complex: implications for schizophrenia and other major neurological disorders. *Mol. Cell. Neurosci.* **25**, 42–55, https://doi.org/10.1016/j.mcn.2003.09.009

76 Atkin, T.A., Brandon, N.J. and Kittler, J.T. (2012) Disrupted in Schizophrenia 1 forms pathological aggresomes that disrupt its function in intracellular transport. *Hum. Mol. Genet.* **21**, 2017–2028, https://doi.org/10.1093/hmg/dds18

77 Kamiya, A., Tomoda, T., Chang, J., Takaki, M., Zhan, C., Morita, M. et al. (2006) DISC1-NDEL1/NDEL protein interaction, an essential component for neurite outgrowth, is modulated by genetic variations of DISC1. *Hum. Mol. Genet.* **15**, 3313–3323, https://doi.org/10.1093/hmg/ddl407

78 Tang, W., Threwdhasan, J.V., Lin, Q., Lim, K.B., Kuroda, K., Kaibuchi, K. et al. (2016) Stimulation of synaptic vesicle exocytosis by the mental disease gene DISC1 is mediated by N-type voltage-gated calcium channels. *Front. Syn. Neurosci.* **8**, 15, https://doi.org/10.3389/fnysn.2016.00015
97 Flores, III, R., Hirota, Y., Armstrong, B., Sawa, A. and Tomoda, T. (2011) DISC1 regulates synaptic vesicle transport via a lithium-sensitive pathway. Neurosci. Res. 71, 71–77, https://doi.org/10.1016/j.neures.2011.05.014

98 Faulkner, R.L., Jang, M.H., Liu, X.B., Duan, X., Sailor, K.A., Kim, J.Y., et al. (2008) Development of hippocampal mossy fiber synaptic outputs by new

99 Duan, X., Chang, J.H., Ge, S., Faulkner, R.L., Kim, J.Y., Kitabatake, Y., et al. (2007) Disrupted-In-Schizophrenia 1 regulates integration of newly

100 Brandon, N.J., Millar, J.K., Korth, C., Silve, H., Singh, K.K. and Sawa, A. (2009) Understanding the role of DISC1 in psychiatric disease and during

101 Taya, S., Shinoda, T., Tsuboi, D., Asaki, J., Nagai, K., Hikita, T., et al. (2007) DISC1 regulates the transport of the NUDEL/LIS1/14-3-3epsilon complex

102 Kojima, M., Shimazaki, H., Iwaya, K., Nakamura, T., Kawachi, H., Ichikawa, K., et al. (2017) Intramucosal colorectal carcinoma with invasion of the

103 Porteous, D. (2008) Genetic causality in schizophrenia and bipolar disorder: out with the old and in with the new. Curr. Opin. Genet. Dev. 18, 229–234, https://doi.org/10.1016/j.gde.2008.07.005

104 Wang, Q., Charych, E.I., Pulito, V.L., Lee, J.B., Graziane, N.M., Crozier, R.A., et al. (2011) The psychiatric disease risk factors DISC1 and TNIK interact to

105 Wei, J., Graziane, N.M., Wang, H., Zhong, P., Wang, Q., Liu, W., et al. (2014) Regulation of N-methyl-D-aspartate receptors by disrupted-in-schizophrenia-1. Biol. Psychiatry 75, 414–424, https://doi.org/10.1016/j.biopsych.2013.06.009
106 Tsuboi, D., Kuroda, K., Tanaka, M., Namba, T., Iizuka, Y., Taya, S. et al. (2015) Disrupted-in-schizophrenia 1 regulates transport of ITPR1 mRNA for synaptic plasticity. Nat. Neurosci. 18, 698–707, https://doi.org/10.1038/n.3984
107 Kanai, Y., Doi, N. and Hirokawa, N. (2004) Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. Neuron 43, 513–525, https://doi.org/10.1016/j.neuron.2004.07.022
108 Sharp, A.H., McPherson, P.S., Dawson, T.M., Aoki, C., Campbell, K.P. and Snyder, S.H. (1993) Differential immunohistochemical localization of inositol 1,4,5-trisphosphate- and ryanodine-sensitive Ca2+ release channels in rat brain. J. Neurosci. 13, 3051–3063, https://doi.org/10.1523/JNEUROSCI.13-07-03051.1993
109 Fitzpatrick, J.S., Hagenston, A.M., Hertle, D.N., Gips, K.E., Bertetto-D’Angelo, L. and Yeckel, M.F. (2009) Inositol-1,4,5-trisphosphate receptor-mediated Ca2+ waves in neuronal dendrites propagate through hot spots and cold spots. J. Physiol. 587, 1439–1459, https://doi.org/10.1113/jphysiol.2009.188930
110 Manita, S. and Ross, W.N. (2009) Synaptic activation and membrane potential changes modulate the frequency of spontaneous elementary Ca2+ release events in the dendrites of pyramidal neurons. J. Neurosci. 29, 7833–7845, https://doi.org/10.1523/JNEUROSCI.0573-09.2009
111 Sala, C., Roussignol, G., Meldolesi, J. and Fagni, L. (2005) Key role of the postsynaptic density scaffold proteins Shank and Homer in the functional architecture of Ca2+ homeostasis at dendritic spines in hippocampal neurons. J. Neurosci. 25, 4587–4592, https://doi.org/10.1523/JNEUROSCI.4822-04.2005
112 Nakamura, T., Lasser-Ross, N., Nakamura, K. and Ross, W.N. (2002) Spatial segregation and interaction of calcium signalling mechanisms in rat hippocampal CA1 pyramidal neurons. J. Physiol. 543, 465–480, https://doi.org/10.1113/jphysiol.2002.020362
113 Matias, C., Dionisio, J.C. and Quinta-Ferreira, M.E. (2002) Thapsigargin blocks STP and LTP related calcium enhancements in hippocampal CA1 area. Neuroreport 13, 2577–2580, https://doi.org/10.1097/00001208-200212200-00039
114 Kuroda, K., Yamada, S., Tanaka, M., Iizuka, M., Yano, H., Mori, D. et al. (2011) Behavioral alterations associated with targeted disruption of exons 2 and 3 of the Disc1 gene in the mouse. Hum. Mol. Genet. 20, 4666–4683, https://doi.org/10.1093/hmg/ddr400
115 Bramham, C.R. and Wells, D.G. (2007) Dendritic mRNA: transport, translation and function. Nat. Rev. Neurosci. 8, 776–789, https://doi.org/10.1038/nrn2150
116 Norkett, R., Modi, S., Birsa, N., Atkin, T.A., Ivanovkic, D., Pathania, M. et al. (2016) DISC1-dependent regulation of mitochondrial dynamics controls the morphogenesis of complex neuronal dendrites. J. Biol. Chem. 291, 613–629, https://doi.org/10.1074/jbc.M115.699447
117 Atkin, T.A., MacAeikll, A.F., Brandon, N.J. and Kitter, J.T. (2011) Disrupted in Schizophrenia-1 regulates intracellular trafficking of mitochondria in neurons. Mol. Psychiatry 16, 122–124, 1, https://doi.org/10.1038/mp.2010.110
118 Murphy, L.C. and Millar, J.K. (2017) Regulation of mitochondrial dynamics by DISC1, a putative risk factor for major mental illness. Schizophr. Res. 187, 55–61, https://doi.org/10.1016/j.schres.2016.12.027
119 Raznahan, A., Lee, Y., Long, R., Greenstein, D., Clasen, L., Addington, A. et al. (2011) Common functional polymorphisms of DISC1 and cortical maturation in typically developing children and adolescents. Mol. Psychiatry 16, 917–926, https://doi.org/10.1038/mp.2010.72
120 Burdick, K.E., Kamiya, A., Covill-Cooke, C., Ivankovic, D., Melandri, D. et al. (2019) Miro clusters regulate ER-mitochondria contact sites and link cristae organization to the mitochondrial transport machinery. Nat. Commun. 10, 4399, https://doi.org/10.1038/s41467-019-12382-4
121 Park, C., Lee, S.A., Hong, J.H., Suh, Y., Park, S., Suh, B.K. et al. (2016) Disrupted-in-schizophrenia 1 (DISC1) and Syntaphilin collaborate to modulate axonal mitochondrial anchoring. Mol. Brain 9, 69, https://doi.org/10.1186/s13041-016-0250-2
122 Courchet, J., Lewis, Jr., T.L., Lee, S., Courchet, V., Liou, D.Y., Aizawa, S. et al. (2013) Terminal axon branching is regulated by the LKB1-NUAK1 kinase pathway via presynaptic mitochondrial capture. Cell 153, 1510–1525, https://doi.org/10.1016/j.cell.2013.05.021
123 Raturi, A. and Simmen, T. (2013) Where the endoplasmic reticulum and the mitochondrion tie the knot: the mitochondria-associated membrane (MAM). Biochim. Biophys. Acta 1833, 213–222, https://doi.org/10.1016/j.bbamcr.2012.04.013
124 Park, S.J., Lee, S.B., Suh, Y., Kim, S.J., Lee, N., Hong, J.H. et al. (2017) DISC1 modulates neuronal stress responses by gate-keeping ER-mitochondria Ca. Cell Rep. 21, 2748–2759, https://doi.org/10.1016/j.celrep.2017.11.043
125 Seshadri, S., Faust, T., Ishizuka, K., Delevich, K., Chung, Y., Kim, S.H. et al. (2015) Interv neuronal DISC1 regulates NRG1-ErbB4 signalling and excitatory-inhibitory synapse formation in the mature cortex. Nat. Commun. 6, 10118, https://doi.org/10.1038/ncomms10118
126 Lepore, D.M., Martinez-Nunez, L. and Munson, M. (2018) Exposing the elusive exocyst structure. Trends Biochem. Sci. 43, 714–725, https://doi.org/10.1016/j.tibs.2018.06.012
127 Park, S.J., Jeong, J., Park, Y.U., Park, K.S., Lee, H., Lee, N. et al. (2015) Disrupted-in-schizophrenia-1 (DISC1) regulates endoplasmic reticulum calcium dynamics. Sci. Rep. 5, 8694, https://doi.org/10.1038/srep08694
128 Shin, D.M., Zhao, X.S., Zeng, W., Mozhaeva, M. and Muallem, S. (2000) The mammalian Sec6/8 complex interacts with Ca(2+) signaling complexes and regulates their activity. J. Cell Biol. 150, 1101–1112, https://doi.org/10.1083/jcb.150.5.1101
129 Emili, F.E., Sedlak, T.W. and Sawa, A. (2014) Oxidative stress and schizophrenia: recent breakthroughs from an old story. Curr. Opin. Psychiatry 27, 185–190, https://doi.org/10.1097/YCO.0000000000000054
130 Ferreira, M.A., O’Donovan, M.C., Meng, Y.A., Jones, J.R., Rudferfer, D.M., Jones, L. et al. (2008) Collaborative genome-wide association analysis supports a role for NK3 and CACNA1C in bipolar disorder. Nat. Genet. 40, 1056–1058, https://doi.org/10.1038/ng.209
131 Green, E.K., Grozeva, D., Jones, I., Jones, L., Kirov, G., Caesar, S. et al. (2010) The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. Mol. Psychiatry 15, 1016–1022, https://doi.org/10.1038/mp.2009.49
162 Belgardt, B.F., Okamura, T. and Brüning, J.C. (2009) Hormone and glucose signalling in POMC and AgRP neurons. *J. Physiol.* **587**, 5305–5314, https://doi.org/10.1113/jphysiol.2009.179192

163 Miesenböck, G., De Angelis, D.A. and Rothman, J.E. (1998) Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins. *Nature* **394**, 192–195, https://doi.org/10.1038/28190

164 Sankaranarayanan, S., De Angelis, D., Rothman, J.E. and Ryan, T.A. (2000) The use of pHluorins for optical measurements of presynaptic activity. *Biophys. J.* **79**, 2199–2208, https://doi.org/10.1016/S0006-3495(00)76468-X

165 Li, H., Foss, S.M., Dobryy, Y.L., Park, C.K., Hires, S.A., Shaner, N.C. et al. (2011) Concurrent imaging of synaptic vesicle recycling and calcium dynamics. *Front. Mol. Neurosci.* **4**, 34, https://doi.org/10.3389/fnmol.2011.00034