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DISC1 regulates N-methyl-D-aspartate receptor dynamics: abnormalities induced by a Disc1 mutation modelling a translocation linked to major mental illness

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Abstract

The neuromodulatory gene DISC1 is disrupted by a t(1;11) translocation that is highly penetrant for schizophrenia and affective disorders, but how this translocation affects DISC1 function is incompletely understood. N-methyl-D-aspartate receptors (NMDAR) play a central role in synaptic plasticity and cognition, and are implicated in the pathophysiology of schizophrenia through genetic and functional studies. We show that the NMDAR subunit GluN2B complexes with DISC1-associated trafficking factor TRAK1, while DISC1 interacts with the GluN1 subunit and regulates dendritic NMDAR motility in cultured mouse neurons. Moreover, in the first mutant mouse that models DISC1 disruption by the translocation, the pool of NMDAR transport vesicles and surface/synaptic NMDAR expression are increased. Since NMDAR cell surface/synaptic expression is tightly regulated to ensure correct function, these changes in the mutant mouse are likely to affect NMDAR signalling and synaptic plasticity. Consistent with these observations, RNASeq analysis of the translocation carrier-derived human neurons indicates abnormalities of excitatory synapses and vesicle dynamics. RNASeq analysis of the human neurons also identifies many differentially expressed genes previously highlighted as putative schizophrenia and/or depression risk factors through large-scale genome-wide association and copy number variant studies, indicating that the translocation triggers common disease pathways that are shared with unrelated psychiatric patients. Altogether, our findings suggest that translocation-induced disease mechanisms are likely to be relevant to mental illness in general, and that such disease mechanisms include altered NMDAR dynamics and excitatory synapse function. This could contribute to the cognitive disorders displayed by translocation carriers.

Introduction

N-methyl-D-aspartate receptors (NMDAR) are vital for synaptic plasticity and cognitive processes, and are strongly implicated in the pathophysiology of schizophrenia in particular. GRIN2A, encoding the NMDAR GluN2A subunit,
was recently reported to show genome-wide significant association with schizophrenia, while genomic copy number variants (CNVs) enriched in schizophrenia patients target GRIN1, encoding the GluN1 subunit. Such findings support a role for NMDAR in psychiatric disorders, but direct mechanistic insight is still required.

NMDAR function is regulated at many stages, including subunit expression and composition, and dynamic modulation of surface and synaptic levels. The latter can be elicited through control of NMDAR forward trafficking to the plasma membrane, subsequent insertion into synapses, or endocytosis. The obligatory GluN1 subunit is incorporated into all NMDAR, along with other subunit types including GluN2A/B. GluN1 is synthesised in excess and stored within the endoplasmic reticulum (ER), where NMDAR are assembled prior to transportation to the Golgi and onwards to the cell surface. Every stage of NMDAR forward trafficking is tightly modulated to ensure that the required quantity of receptors is present at the cell surface and synapse. Genetic dysregulation of forward trafficking would be predicted to adversely affect the NMDAR function, with knock-on effects for synapse strength and plasticity.

DISC1 is disrupted in a large, multi-generation family by a chromosomal t(1;11) translocation that is linked to major mental illness. DISC1 is critical for several processes in the developing and adult brain, and has been connected to NMDAR function through regulation of downstream processes that underlie synaptic plasticity. DISC1 also regulates neuronal microtubule-based cargo transport, including trafficking of mitochondria, synaptic vesicles and messenger RNAs, and associates with the motor protein adaptors TRAK1 and TRAK2.

Here we demonstrate direct interaction between GluN1 and DISC1, and association between GluN2B and TRAK1. Applying a novel live-imaging method, we demonstrate that DISC1 regulates dendritic NMDAR motility. Utilising a mouse model of DISC1 disruption by the translocation, we show that mutant mouse neurons exhibit increased NMDAR fast active transport and cell surface/synaptic NMDAR expression. These observations implicate dys-regulated NMDAR dynamics/signalling and excitatory synapse dysfunction in the psychiatric disorders displayed by translocation carriers. In further support of a general disease mechanism revealed by the translocation, we find that it impacts the biological pathways highlighted by independent schizophrenia and depression GWAS and CNV studies.

Materials and methods

Detailed materials and methods for all experiments are provided in the Supplementary information. Essential information is included below.

Generation of induced pluripotent stem cells (iPSC) from dermal fibroblasts

Dermal fibroblasts were reprogrammed using non-integrating episomal plasmids incorporating Oct3/4, shRNA to p53, SOX2, KLF4, L-MYC and LIN28, and converted to neural precursor cells by dual-SMAD signalling inhibition.

Generation of a mouse model of the t(1;11) translocation

Mice were genetically engineered using Regeneron’s GEMM platform (VelociMouse®). Disc1 was targeted in embryonic stem cell clones using a vector containing mouse Disc1 genomic DNA encompassing exons 6, 7 and 8 as the 5’ homologous arm, and sequences 3’ of Disc1 as the 3’ homologous arm. Human chromosome 11 genomic DNA, encompassing putative exons 4, 5, 6, 7a and 7b of DISC1FP1 followed by a loxP-flanked neo cassette, was inserted between the mouse homologous arms. Homologous recombination removed 98,550 bp of mouse Disc1 downstream of exon 8 and inserted 114,843 bp of human DISC1FP1 sequence. The edited endogenous mouse Disc1 locus mimics the Disc1/Disc1FP1 gene fusion event on the derived chromosome 1 in translocation carriers, and the mutation is referred to as Der1.

RNA sequencing

Three control and three translocation-carrying neural precursor lines were each differentiated to neurons independently in triplicate. RNA was extracted and sequenced to a depth of 60 million paired-end reads. Differential gene expression was analysed at the whole gene and single exon levels using DESeq2 from the R statistical package and DEXSeq, respectively.

Dendra2 NMDAR trafficking assay

A Dendra2 tag was fused to the GluN1 C terminus. Dendra2 exhibits green fluorescence, but can be photoconverted to red fluorescence. DIV7 hippocampal neurons were co-transfected with GluN1–Dendra2 plus HA–GluN2B to maximise NMDAR assembly, and therefore trafficking outwith the ER. Dendra2 was photoconverted within a region of interest positioned on a primary dendrite. Time-lapse images were captured every 15 s for 3 min, and red fluorescence bidirectional movement along dendrites was quantified. Essentially, dendrites were divided into 5-μm bins, and red fluorescence intensity was quantified within each bin at each timepoint. Finally, the fluorescence intensity within each bin was normalised to dendritic area and fluorescence intensity in bin zero at time zero. Fluorescence peak average velocities were determined individually for each neuron using the 10-μm and 15-μm bins where the peak was clearly identifiable. Maximum velocity estimates were determined individually for each
neuron using the 25–40-μm bins, where the appearance of the leading edge of the wave of red fluorescence could be ascertained.

Results
Neural precursor cells (NPC) and neurons derived from t (1;11) translocation carriers
Dermal fibroblasts from translocation carriers diagnosed with schizophrenia, recurrent major depression, cyclothymia or recurrent major depression plus bipolar disorder (not otherwise specified) and karyotypically normal family members (Supplementary Figure 1) were reprogrammed using non-integrating episomes18. NPC lines were generated from the IPSC (Supplementary Figure 2a–c) as described previously19. All NPC lines exhibit typical morphology and form rosettes in the culture, with almost 100% of cells expressing the neural progenitor marker NES (Supplementary Figure 2c). Each NPC line consistently differentiates to produce neurons expressing the neuronal marker βIII-tubulin (Supplementary Figure 2d).

To examine the effect of the translocation upon DISC1 transcript levels in the t(1;11) family NPCs and neurons, NPCs were differentiated over 5 weeks, with sampling at time zero (before differentiation onset) and weekly thereafter, to obtain the RNA for quantitative RT-PCR analysis. Wild-type DISC1 expression is decreased in translocation-carrying NPCs and neurons relative to controls (Fig. 1a). Moreover, DISC1 expression increases as differentiation progresses (Fig. 1a, b), although the proportional change in each week is not altered by the translocation (Fig. 1b). DISC1 expression may therefore be particularly important in neurons. An antibody that detects full-length DISC1 (Supplementary Figure 3a) confirmed its reduced expression in NPCs and neurons (Fig. 1c, d).

Chimeric CP1 and CP60/69 transcripts, resulting from translocation-induced DISC1 gene fusion to the DISC1FP1 (otherwise known as Boymaw) gene on the derived chromosome 120, were PCR-amplified from NPCs and neurons derived from translocation carriers (Fig. 1e). These transcripts encode aberrant forms of DISC1, which, when exogenously expressed, are deleterious20, thus they are likely damaging to the brain in vivo. However, their endogenous expression awaits confirmation.

DISC2 and DISC1FP1, also disrupted by the translocation, are expressed in the NPCs and neurons, but at low, non-quantifiable levels (Supplementary Figure 3b).

Transcriptomic analysis of t(1;11) family neurons
We next performed RNA sequencing (RNASeq) analysis as an unbiased screen for the effects of the translocation upon gene expression in the human cortical neurons. The translocation lines used were derived from patients diagnosed with schizophrenia, recurrent major depression or cyclothymia. The use of cross-diagnostic samples aimed to ensure that the major effects of the translocation are identified, rather than any disorder-specific effects due to genetic background. Overall, 1,256 (BaseMean>10) of 22,753 expressed genes were found to be differentially expressed (adjusted p < 0.05, Fig. 2a, Supplementary Table 1a). A total of 938 genes also exhibit differential expression of individual exons (adjusted p < 0.05), an indicator of altered isoform expression (Supplementary Table 1b).

IPSC-derived neuron cultures are a mix of cell types, thus it is possible that the translocation could affect gene expression patterns via influences upon cell fate. However, analysis of genes whose expressions were determined to be enriched in mouse or human astrocytes and neurons by enriched cell or single-cell RNASeq analysis20,21 found that only 7/41 mouse and 3/21 human astrocyte markers, and 8/25 mouse and 5/34 human neuron markers are dysregulated (Supplementary Table 1c, d). Four of the dysregulated mouse neuron markers, DLX1, DLX2, DLX5 and DLX6, are already known to be regulated by DISC1, indicating a subtle shift in neuronal properties22,23. Classic markers of neurons, and of inhibitory GABAergic neurons, are also unaffected (Supplementary Table 1d). Moreover, analysis of genes whose expressions are enriched in specific ‘communities’ of human excitatory and inhibitory neurons21 found little indication of change to either type overall (Supplementary Table 1e). Thus, the translocation does not affect the proportion of astrocytes versus neurons, or of excitatory versus inhibitory neurons, although neuron properties may be subtly altered. This is consistent with a previous study which examined the effects of a DISC1 mutation upon gene expression in IPSC-derived neurons22.

A total of 300 and 71 of the dysregulated genes, respectively, are also differentially expressed in IPSC-derived neurons carrying a frameshift mutation in DISC1 exon 1225 (hypergeometric probability p = 2.5E-21 Supplementary Table 1a, b) or exon 822 (p = 0.00002), indicating that independent DISC1 mutations have overlapping effects. Differential DISC1 expression was not initially detected in the whole-gene analysis, presumably due to the presence of chimeric DISC1 transcripts originating from both derived chromosomes (Fig. 1e, Supplementary Figure 3c). However, comparison of reads that span intron 8, the location of the translocation breakpoint and that can therefore only arise from wild-type transcripts, indicates that wild-type DISC1 transcripts are indeed reduced (Fig. 2b).

Due to the potential for positional, or other, effects of the translocation upon neighbouring gene expression, we examined the transcript levels over a region of approximately 20 Mb proximal and distal to the translocation breakpoints on chromosomes 1 and 11. Such analysis
Fig. 1 (See legend on next page.)
identified a number of differentially expressed genes and exons, and possible clustering of dysregulated genes around the chromosome 11 breakpoint (Supplementary Table 1f, g). Gene ontology (GO) analysis (Fig. 2c) highlighted cell/neuron projections (seven of the top 20 GO Component terms), the post-synaptic density of excitatory synapses (2/20) and vesicles/organelles (4/20). Additional significant GO Process and GO Function terms relate to synapse assembly, cell adhesion and the actin cytoskeleton. Ingenuity pathway analysis (IPA), a complementary method, identified similar terms, including terms predicting that the translocation will affect synapse assembly and function in mature neurons of the brain (Fig. 2d). IPA also found that the term ‘movement of organelle’ was significant (Fig. 2d). Given that the RNAseq analysis is unbiased, it is notable that DISC1 regulates processes related to at least three of the major enriched categories; neurite extension, trafficking and actin remodelling. We therefore infer that these process-related gene expression changes are most likely due to DISC1 disruption.

While the translocation is unique to a single family, translocation carriers do not present unusual clinical symptoms outside the current diagnostic criteria, thus the consequences of the translocation may converge upon biological processes shared with other unrelated patients. In support of this, several of the differentially expressed genes in t(1;11) neurons correspond to genome-wide association study (GWAS) findings for schizophrenia. Expression of 33 of the 348 genes from 25 of the 108 associated loci (hypergeometric probability $p = 7E-6$) indicates enrichment for loci containing at least one dysregulated gene) identified in the first study, and 37 of the 481 genes from 24 of the 145 loci (hypergeometric probability $p = 0.001$) identified in the second study is altered at the whole-gene and/or exon level in control versus translocation lines (Supplementary Table 1a, b). Of these dysregulated putative schizophrenia genes, changed expressions of DRD2, encoding the dopamine D2 receptor, a target of all antipsychotics in clinical use, and PDE4B, encoding a CAMP-degrading phosphodiesterase and known DISC1 interactor, were confirmed by quantitative RT-PCR (Fig. 2e). Altered expression was also confirmed for genes encoding the historical candidate NRG1 and its receptor ERBB4 (Fig. 2e), both of which have previously been linked functionally to DISC1. Expression of 12 of 52 genes encoding synaptic proteins that are targeted by recurrent CNVs in schizophrenia is also altered (hypergeometric probability $p = 0.001$, Supplementary Table 1a, b). Four of 70 genes at four of 44 depression-associated loci (9.1% of loci, no enrichment) identified through GWAS are also dysregulated (Supplementary Table 1a, b).

Finally, three of four recently identified putative modifier loci in the t(1;11) family are adjacent to the translocation breakpoints on chromosomes 1 or 11 (Supplementary Table 1f, g). Of the three genes of interest identified therein, GRM5 is not dysregulated in the human cortical neurons (Fig. 2e, Supplementary Table 1a, b), CAPN8 is expressed at very low, non-quantifiable levels and CNTN5 is dysregulated according to the RNASeq analysis ($p = 0.0008$, Supplementary Table 1a, b). However, although quantitative RT-PCR also suggests increased CNTN5 expression, this did not achieve statistical significance (Fig. 2e). The fourth locus, encompassing PDE4D, could not be examined because the cell lines used for RNASeq analysis and quantitative RT-PCR do not carry the required haplotype.

A mutant mouse recapitulates the effect of the t(1;11) translocation upon DISC1

A mutant mouse was generated by deleting genomic DNA containing Disc1 exons 9–13 and replacing it with human chromosome 11 genomic DNA containing relevant DISC1FP1 exons (Fig. 1f). This mimics the gene fusion caused by the translocation on the derived chromosome 1, thus we refer to the mutation as Der1.

Quantitative RT-PCR demonstrated that wild-type Disc1 transcript expression is reduced by approximately
Fig. 2 (See legend on next page.)
half in whole brain isolated from adult heterozygous Der1 (Disc1Der1/Der1) mice in comparison with wild-type (Disc1Der1/Der1) mice (Fig. 1g). Full-length Disc1 protein expression is detected at ~100 kDa, and is correspondingly reduced in Disc1Der1/Der1 versus Disc1Der1/Der1 mice (Fig. 1h). As expected, wild-type Disc1 transcripts and protein were undetectable in whole brain from homozygous (Disc1Der1/Der1) mice (data not shown, Fig. 1h). CP60/69 transcripts were found to bind directly to GluN1 peptide spots 1–16, encompassing the alternatively spliced C0, C1 and C2 cassettes. We next used FLAG-DISC1 to probe peptide arrays of DISC1 amino acids 361–385 was interrogated with the same GluN1 probe (Fig. 3e), revealing that binding to the wild-type sequence is substantially weakened by alanine conversion of any one of the amino acids E374, L380, E384, D382, L383, E384 and Q385. The DISC1 head domain therefore contacts GluN1 via a single site in which residues 380–385 are critical for binding. This binding site was independently confirmed by co-immunoprecipitating HA–GluN1 and GST-DISC1 amino acids 358–499 from COS7 cells (Fig. 3f). Moreover, exogenous DISC1 co-localises with endogenous GluN1 in cultured DIV8 and DIV14 hippocampal neurons (Fig. 3g). These observations indicate that DISC1 and the putative schizophrenia risk factor GluN13 have the capacity to interact directly in vivo.

**TRAK1 complexes with GluN2B**

Because DISC1 interacts with GluN1 and regulates cargo transport, we speculated that DISC1 and TRAK1 might be involved in NMDAR trafficking. To test this, we co-transfected COS7 cells with TRAK1 plus NMDAR subunit, GluN1 or GluN2B expression constructs. In the absence of TRAK1, both GluN1 and HA–GluN2B predominantly co-localise with the ER marker Calreticulin (Fig. 3h), while FLAG-TRAK1 is strongly targeted to the mitochondria, as expected17 (Fig. 3i). In co-transfected cells, there is no appreciable co-localisation between FLAG-TRAK1 and GluN1 (Fig. 3i), but in the presence of FLAG-TRAK1, HA–GluN2B redistributes from the ER to the mitochondria (Fig. 3i). Moreover, in triple-transfected cells, in the presence of HA–GluN2B plus FLAG-TRAK1, a substantial proportion of GluN1 localises in the mitochondria (Fig. 3j), indicating that assembled NMDAR can associate with TRAK1. We next immunoprecipitated Trak1 from mouse brain synaptosomal and light membrane fractions, where both proteins are enriched33 (data not shown), and demonstrated co-precipitation of GluN2B (Fig. 3k, Supplementary Figure 4). The DISC1-associated trafficking factor Trak1 thus associates with the NMDAR GluN2B subunit in vivo. Disc1, with TRAK1, is now well established as a regulator of neuronal intracellular trafficking34. The GluN1/DISC1/TRAK1 and TRAK1/GluN2B associations therefore imply that DISC1 regulates the motility of GluN2B-containing NMDAR.
Fig. 3 (See legend on next page.)
DISC1 dysregulates dendritic GluN1–Dendra2 motility in mouse hippocampal neurons

To examine NMDAR motility in neurons, we fused the green fluorescent protein Dendra2 to GluN1, which is incorporated into all NMDAR. Dendritic GluN1–Dendra2 has a granular appearance similar to that of endogenous GluN1 (Fig. 4a, Supplementary Figure 5a)\cite{35}. Dendra2 can be stably photoconverted to red fluorescence, enabling tracking of a specific red GluN1 population (Supplementary Video). Dendra2 fusion to the GluN1 C terminus does not interfere with the receptor assembly or trafficking to synapses because GluN1–Dendra2 co-localises with the post-synaptic density marker PSD95 within dendritic spines (Supplementary Figure 5b). This can only occur following proper receptor assembly\cite{5}.

Bulk GluN1–Dendra2 movement was quantified in cultured wild-type hippocampal neurons expressing exogenous human DISC1 at days in vitro 8 (DIV8), an age at which the dendritic arbour is suitably developed, but not too complex. Following GluN1–Dendra2 photoconversion, red fluorescence bidirectional spreading was imaged and analysed every 15 s for a total of 2 min in successive 5–20-μm bins along the dendrites (Supplementary Figure 5c–n).

We examined the effects of wild-type DISC1 and a change of arginine to tryptophan at position 37 (37W) in DISC1 (Supplementary Figures 6a–f, 7a, b). This sequence variant has been reported in four individuals, all with a diagnosis of psychiatric illness\cite{36,37}, and dysregulates DISC1/TRAK1 association\cite{17}. Wild-type DISC1 overexpression increases distal-moving red fluorescence in the 20–35-μm bins (Supplementary Figure 6c, d), while in the proximal direction, it reduces red fluorescence intensity in all bins (Supplementary Figure 7a, b). In contrast, DISC1–37W overexpression increases and decreases distal-moving red fluorescence in the 5–15-μm and 25–35-μm bins, respectively (Supplementary Figure 6c, d), while proximal-moving red fluorescence increases in the 5–20-μm bins (Supplementary Figure 7a, b).

These data are consistent with the existence of at least two classes of distal-moving dendritic GluN1–Dendra2 in young hippocampal neurons that are differentially influenced by DISC1: (1) a slow-moving population in the 5–15/20-μm bins (average velocity = 0.2 μm/s in empty vector-transfected neurons, Supplementary Figures 6d, e, 7b), representing the majority of the GluN1–Dendra2 and (2) a fast-moving population in the 25–35-μm bins (average velocity = 0.82 μm/s in empty vector-transfected neurons, Supplementary Figures 6d, f, 7b).

These populations represent the trailing edge plus ‘crest’ and the leading edge, respectively, of a wave of motile GluN1–Dendra2 (Supplementary Figures 6d, 7b). Our evidence for these populations aligns well with estimates of 0.2–0.3μm/s for the movement of dendritic ER vesicles\cite{38} and with estimates of 0.76 μm/s for the motility rate of assembled NMDAR-containing vesicles undergoing fast active transport in dendrites\cite{39}, thus the slow and fast populations likely represent the ER GluN1 pool and NMDAR-containing vesicles undergoing active transport, respectively.

Altered dendritic NMDAR motility in Der1 mouse hippocampal neurons

We next quantified bulk GluN1–Dendra2 movement within neuronal dendrites from Disc1<sup>wt/wt</sup> and Disc1<sup>Der1/</sup>Der1 mice using the same method (Fig. 4b–g, Supplementary Figure 7c, d). The same two pools of GluN1 were detected, with the distal-moving ER and transport vesicle pools, respectively, decreased and increased in Disc1<sup>Der1/</sup>Der1 mutant neurons (Fig. 4d, e). A similar, but less marked, pattern of proximal GluN1–Dendra2 motility was observed (Supplementary Figure 7c, d).

Altered dendritic NMDAR surface distribution in Der1 mouse hippocampal neurons

The NMDAR trafficking data indicate that DISC1 modulates NMDAR motility and consequently predict dysregulated cell surface NMDAR expression in neurons.
average velocities:
WT (proximal)=0.27 μm/s, WT (distal)=0.27 μm/s
HOM (proximal)=0.21 μm/s, HOM (distal)=0.24 μm/s

average velocities:
WT (proximal)=0.76 μm/s, WT (distal)=0.74 μm/s
HOM (proximal)=0.68 μm/s, HOM (distal)=0.64 μm/s
cultured from the Der1 mutant mouse. To test this, we quantified the NMDAR cell surface expression in DIV21 cultured Disc1wt/wt, Disc1wt/Der1 and Disc1Der1/Der1 hippocampal neurons, an age at which neurons have matured and developed synapses. Cell surface expression of the NMDAR subunits GluN1, GluN2A and GluN2B and of the post-synaptic density marker PSD95 and βIII-tubulin (TuJ1) was imaged at high resolution by three-dimensional structured illumination microscopy (3D-SIM, Supplementary Figure 8a), which achieved a lateral image resolution of 180 nm for the NMDAR subunits and 120 nm for PSD95.

3D reconstructions of βIII-tubulin and GluN1, GluN2A and GluN2B were used to estimate dendritic volume and the number and volume of individual surface puncta of each NMDAR subunit, respectively (Fig. 5a, b, Supplementary Table 2). This analysis demonstrated increased density of GluN1 and GluN2B puncta in Disc1wt/Der1 and Disc1Der1/Der1 mutant neurons, while GluN2A puncta density is increased only in Disc1Der1/Der1 mutant neurons. Total surface expression is correspondingly increased in Disc1wt/Der1 and Disc1Der1/Der1 mutant neurons. Average puncta volumes are increased for all three NMDAR subunits, but only in Disc1wt/Der1 neurons, suggesting that NMDAR form larger clusters on the cell surface in heterozygous neurons. This observation supports the existence of putative aberrant proteins encoded by the putative determinant of excitatory synapse function and neurotransmission. Expression of PSD95 was therefore quantified in the imaged set of hippocampal neurons to determine whether synapse size and structure is also affected by Der1 mutation.

Super-resolution microscopy has demonstrated the existence of varying numbers of PSD95 puncta (nanodomains) at the post-synaptic density, NMDAR stabilisation at synapses, assembly of NMDAR-associated signalling complexes and recruitment of AMPA receptors (AMPA), the major excitatory receptors in the brain. PSD95 is thus a major determinant of excitatory synapse function and neurotransmission. Expression of PSD95 was therefore quantified in the imaged set of hippocampal neurons to determine whether synapse size and structure is also affected by Der1 mutation.

Altered distribution of PSD95 in Der1 mouse hippocampal neurons

PSD95 is one of the most abundant molecular scaffolds at the post-synaptic density of excitatory synapses. It has many functions including organisation of the post-synaptic density, NMDAR stabilisation at synapses, assembly of NMDAR-associated signalling complexes and recruitment of AMPA receptors (AMPA), the major excitatory receptors in the brain. PSD95 is thus a major determinant of excitatory synapse function and neurotransmission. Expression of PSD95 was therefore quantified in the imaged set of hippocampal neurons to determine whether synapse size and structure is also affected by Der1 mutation.

At the neuronal cell surface NMDAR are present at synaptic, perisynaptic and extrasynaptic sites, thus only a proportion of receptors co-localise with PSD95 at synapses. Applying Pearson’s co-localisation coefficient, which quantifies the overall correlation between two fluorescent signals, indicates that PSD95 co-localisation with GluN1 is substantially increased in Disc1Der1/Der1 mutant neurons (Fig. 5c, d). However, synaptic localisation of GluN2A and GluN2B is not greatly altered (Supplementary Figure 9a, b). Since GluN1 subunits cannot be transported to synapses unless they have assembled with other subunit types, we suggest that this discrepancy is related to the presence of two GluN1 subunits in every NMDAR, with variable inclusion of other subunit types, resulting in a clear effect for the former, but not for the latter. If correct, the GluN1/PSD95 co-localisation data indicate that NMDAR are more abundant at synapses in the Disc1Der1/Der1 mutant neurons.

Hippocampus protein extract immunoblotting demonstrated that total expression of GluN1, GluN2A and GluN2B is unaltered by mutation in adult Disc1wt/Der1 or Disc1Der1/Der1 mice (Supplementary Figure 9c), confirming that surface expression changes are due to altered NMDA receptor subunit distribution.

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Fig. 5 (See legend on next page.)
motility is in cell surface and synaptic NMDAR expression. NMDAR within dendrites, a process that is critical for controlling as for schizophrenia. (1;11)-carrying human neurons, although not as strikingly prevalent in t(1;11) carriers, and we found differential expression in these studies are differentially expressed in human neurons carrying a t(1;11) linked to major mental illness. Moreover, the gene DISC1, disrupted by the t(1;11), encodes a direct interactor of the NMDAR obligatory GluN1 subunit. This convergence of the t(1;11) with schizophrenia2–4,24. We show here that multiple genes identified in these studies have an increased density of single nanodomains (Fig. 6c), and of small nanodomain clusters of 2–3 (Fig. 6d).

Discussion
Recent large-scale GWAS and CNV studies implicate excitatory synapses, NMDAR subunits GluN1 and GluN2A, and synaptic plasticity as causal factors in schizophrenia2–4,24. We show here that multiple genes identified in these studies are differentially expressed in human neurons carrying a t(1;11) linked to major mental illness. Moreover, the gene DISC1, disrupted by the t(1;11), encodes a direct interactor of the NMDAR obligatory GluN1 subunit. This convergence of the t(1;11) with schizophrenia risk genes indicates that the t(1;11) is a trigger for disease pathways, which is shared with unrelated schizophrenia patients. Affective disorders are also prevalent in t(1;11) carriers, and we found differential expression of putative depression risk factors in the t(1;11)-carrying human neurons, although not as strikingly as for schizophrenia.

We demonstrate that DISC1 regulates NMDAR motility within dendrites, a process that is critical for controlling cell surface and synaptic NMDAR expression. NMDAR motility is influenced by a DISC1–37W mutation36,37, previously shown to affect mitochondrial motility47, and by the mouse Der1 mutation which accurately models the effect of the t(1;11) upon DISC1 expression. DISC1, DISC1–37W and the Der1 mutation influence the size of the GluN1 ER pool, indicating, in the absence of altered NMDAR subunit expression, that DISC1 regulates the NMDAR ER-exit. Indeed DISC1 is ER-associated48, and may directly impact NMDAR ER-exit because the binding site for DISC1 on GluN1 encompasses two ER retention signals (Fig. 3b)5. However, a simple model whereby increased/decreased ER-exit leads to, respectively, decreased/increased transport pool size cannot fully explain the effects of DISC1 upon NMDAR motility due to the lack of an overt relationship between the observed sizes of the ER and active transport pools. We therefore propose that DISC1 influences the active transport pool independently of ER-exit via TRAK1, which associates with the NMDAR subunit GluN2B, and its role as a regulator of neuronal cargo trafficking48.

Mouse neurons expressing DISC1–37W exhibit a decreased pool of NMDAR undergoing fast active transport, while Der1 mutant mouse neurons exhibit an increased pool. Since the 37 W variant has to date been found only in psychiatric patients, this suggests that dysregulation of this pool, up or down, is deleterious. Upregulation of the pool in Der1 mutant mouse neurons correlates strikingly with the augmented density and overall volume of cell surface NMDAR clusters, suggesting that increased trafficking/membrace insertion largely account for the greater surface NMDAR volume. NMDAR are constantly exchanged between synaptic and extrasynaptic sites45, with synaptic insertion events believed to result from stochastic post-synaptic density-mediated capture of receptors undergoing lateral diffusion within the cell membrane46. Consequently, the elevated NMDAR surface volume in Der1 mutant mouse neurons likely increases the probability of their diffusion to, and insertion into, synapses, thus explaining the increased synaptic localisation of GluN1.

In addition to the NMDAR abnormalities, the post-synaptic density scaffold PSD95 is aberrantly distributed in Der1 mutant mouse neurons. Post-synaptic density size at excitatory synapses is directly related to the number of PSD95 nanodomains present43 and to AMPAR expression and synapse size/strength44. Moreover, the number of PSD95 nanodomains at a synapse is proposed to be directly related to the number of AMPAR at a synapse42,44,47. Synaptic PSD95 nanodomain number therefore likely predicts the synaptic AMPAR expression and in turn, synapse strength, which is determined by the
number of AMPAR present. On this basis, the Der1 mutant mouse neurons are predicted to possess fewer synaptic AMPARs and weaker synapses.

AMPAR-induced depolarisation initiates NMDAR-dependent synaptic plasticity and long-term potentiation via molecular pathways involving signalling molecules such as CAMKII, PKA, PDE4 and the transcription factor CREB\(^{48–52}\). This leads to increased synaptic AMPAR expression and dendritic spine volume through actin remodelling, and thus greater synapse strength and size\(^{53,54}\). Various DISC1 mutant mice exhibit synaptic plasticity defects\(^{34}\) and in some cases involvement has been demonstrated of PDE4, which interacts with and is regulated by DISC1\(^{25}\) or CREB\(^{34}\). Moreover, DISC1 is known to regulate the AMPAR subunit expression and dendritic spine size/density downstream of NMDA receptors via actin remodelling\(^{11,55}\), and could therefore control plasticity through these mechanisms. DISC1 is also reported to regulate the NMDAR activity through PDE4-mediated activation of CREB-dependent GluN2A expression\(^{12}\), although we found no evidence of this mechanism in Der1 mice since hippocampal GluN2A levels are unaltered. DISC1 may therefore regulate synaptic plasticity at multiple levels downstream of NMDAR, and it is notable that the gene expression changes in the human neurons derived from t(1;11) carriers, including altered expression of PDE4B and genes required for actin cytoskeleton remodelling, are a good fit with these processes. Crucially, however, our data indicate that DISC1 has the potential to modulate the triggering of plasticity itself, through effects upon synapse strength and via direct interaction with GluN1, regulation of NMDAR dynamics and thus control of their synaptic expression and availability to initiate plasticity. Synaptic plasticity underlies cognition which is characteristically impaired in schizophrenia in particular, but also in affective disorders. Our findings thus point towards a pathway that is of direct relevance to a core feature of psychiatric disorders in t(1;11) carriers and to major mental illness in general.

Four potential genetic modifiers have recently been identified in the t(1;11) family\(^{30}\). Of the genes-of-interest identified at these loci, only GRM5 and CNTN5 could be meaningfully examined using the selected panel of t(1;11) family neurons and although abundantly expressed,
neither was found to be dysregulated. Nor were any deleterious sequence variants that would affect protein function discovered in these genes\(^{30}\), although potential expression quantitative trait loci (eQTLs) that could affect the GRM5 expression levels were previously identified within the modifier\(^{29}\). Such eQTLs may therefore act upon GRM5 in a cell type-specific manner. The \textit{Der1} mouse mimics disruption of \textit{DISC1} by the t(1;11), isolating this event from any effects of the putative modifiers. We therefore propose that the deleterious effects of \textit{DISC1} disruption upon excitatory synapses, identified here using the \textit{Der1} mutant mouse, contribute substantially to risk of major mental illness in t(1;11) carriers, but can be influenced by the genetic modifiers and/or environmental factors, leading to differing outcomes. This is consistent with a previous observation that, irrespective of diagnosis, all tested t(1;11) carriers exhibit an abnormal schizophrenia endophenotype, the P300 event-related potential\(^{6}\), and that P300 abnormalities are linked to NMDAR dysfunction\(^{56}\).

In conclusion, the \textit{Der1} mutation-induced NMDAR trafficking and synapse abnormalities described here converge upon themes of dysfunctional NMDAR, excitatory synapses and plasticity that are emerging from schizophrenia and depression GWAS and CNV data. The t(1;11) thus provides a mechanistic window into the biochemical basis of these disorders, which links to genetic findings and highlights new ways to consider therapeutic intervention.

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Conflict of interest
The authors declare that they have no conflict of interest.

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