A mutation in mouse Disc1 that models a schizophrenia risk allele leads to specific alterations in neuronal architecture and cognition

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**The endogenous Disc1 orthologue that models the only well defined DISC1 schizophrenia risk allele (2). This approach preserves the endogenous spatial and temporal expression pattern of the gene, thus preventing the induction of neomorphic phenotypic features. A comprehensive analysis of these mice implicates malfunction of neural circuits within the hippocampus (HPC) and medial prefrontal cortex (mPFC), and selective deficits in WM as contributing to the genetic risk conferred by the DISC1 gene.**

**Results**

The mutant mouse strain used in this study propagates a Disc1 allele (Mouse Genome Informatics nomenclature: Disc1/TmirKara) (Fig. 1A) that carries two termination codons (in exons 7 and 8) and a premature polyadenylation site in intron 8, which leads to the production of a truncated transcript (2). Western blot analysis of total brain extracts around the peak of postnatal Disc1 expression using two polyclonal antibodies raised against N-terminal (2) and C-terminal [see supporting information (SI) Fig. S1 A and B] Disc1 protein moieties revealed that the introduced genetic lesion results in the elimination of the major isoforms of Disc1 at ~98-kDa (predicted full-length proteins L and Lc forms http://genome.ucsc.edu) and ~70 kDa (14). It also results in the production of low levels of a predicted truncated protein product detected, as expected, by the N-terminal but not the C-terminal antibody (Fig. 1B). In the adult brain, Disc1 levels are drastically reduced (15). In the adult HPC, the ~70-kDa signal is undetectable, although a WT-specific signal still persists at the 98-kDa range (L and Lc forms). No truncated protein product is observed (Fig. 1B). It should be noted that two commercially available antibodies raised against small peptide epitopes located at the N and C terminal of Disc1 failed to detect elimination of specific WT bands, both at the 98- and 70-kDa range. Moreover, the N-terminal peptide antibody failed to recognize the novel product at the expected size of a truncated Disc1 protein (Fig. S1 C and D). Thus, unlike two polyclonal antibodies raised against extended protein domains, both peptide antibodies demonstrate lack of specificity when used to analyze complex brain extracts, raising questions about the
reliability of these antibodies when probing the pattern of expression of Disc1 isoforms (16) (M. Kvajo, unpublished work). Overall, the genetic lesion introduced into the endogenous Disc1 orthologue models closely the Scottish mutation by virtue of the position of the introduced truncating lesions (in the vicinity of the translocation breakpoint) and of the fact that it preserves short N-terminal isoforms of the gene (http://genome.ucsc.edu). We used this mouse strain to examine in more detail two brain regions (HPC and mPFC) implicated in schizophrenia (17).

**Morphological Analysis of the Adult mPFC.** Low-resolution histology in Nissl-stained sections indicated no gross changes in HPC or mPFC morphology (Fig. S2 A and C). Volumetric analysis showed no genotypic differences in HPC volume (P = 0.89) (Fig. S2B), but revealed a small decrease (14%, P < 0.05) in mPFC volume compared with WT littermate controls (Fig. S2D). By intercrossing mutant Disc1 mice with a reporter strain (GFP-M) (18) we identified a ~10% decrease (P < 0.05) in apical dendrite length of sparsely labeled layer V pyramidal neurons, which corresponds well with the decrease in the volume of this brain area (Fig. S2 E and F). We found no differences in the soma size or the mean angle of orientation (Fig. S2F). The complexity and total length of the basal dendritic tree and the apical tuft (Fig. S2 G–J and data not shown) were also unchanged, suggesting that the impaired extension of the apical dendrite may be caused by noncell autonomous restrictions imposed by the reduced mPFC volume. Finally, we found no differences in the numbers of calbindin- or parvalbumin-positive interneurons (Fig. S3 A and B).

**Cytoarchitectural Alterations in the Adult Dentate Gyrus (DG).** Postnatally, Disc1 expression persists primarily in the DG (15), a brain region with active neurogenesis during adulthood (19). We did not observe any gross changes in DG morphology, and volumetric analysis did not show a significant decrease in the volume of the DG granule cell layer (GCL) in mutant Disc1 mice (P = 0.16) (Fig. S4A and B). In addition, immunocytochemistry with a calbindin antibody did not reveal any gross abnormalities in the mossy fiber tract (Fig. S4A).

**Immature Granule Cells.** In an effort to search for subtler morphological alterations, we first examined the immature neuronal population in the DG by using doublecortin (DCX) immunoreactivity. The subgranular zone (SGZ) of the DG harbors progenitor cells that continuously divide and give birth to new neurons, which migrate into the GCL, extend dendrites, and become integrated into functional circuits (19). In both genotypes, the majority of DCX-positive neurons were found in the SGZ and inner GCL with only a smaller proportion reaching the deeper layers of the GCL (Fig. 2A). However, a higher portion of cells was located in the outer layers of the GCL in mutant Disc1 mice (Fig. 2A, arrows). Analysis of the distance traveled by the furthest migrating cells (20) revealed that a larger fraction of these cells reached the outer layers of the GCL in mutant Disc1 mice (P = 0.024) (Fig. 2B). The maturation of newly born neurons is tightly coupled to their migration, with more mature neurons occupying the outer layers of the GCL (21). No genotypic difference was observed in the number of DCX-positive cells expressing NeuN, a marker of mature neurons, suggesting that the ectopic localization is not a consequence of enhanced maturation (Fig. S4C).

The apical dendrites of most WT DCX-positive cells migrating into the GCL are oriented approximately perpendicular to the SGZ surface. By contrast, apical dendrites in mutant Disc1 mice were often misoriented (Fig. 2C). To quantify this phenotype we measured the direction of each apical dendrite as an angle of orientation (θ) relative to the SGZ surface (22) (Fig. 2D). In WT mice, the mean angle of orientation was ~10° (9.93 ± 1.43, n = 42 cells), whereas in mutant Disc1 mice it was twice as large (19.08 ± 2.22, n = 44 cells, P < 0.005) (Fig. 2E). In WT mice, ~7% of tested young neurons had apical dendrites projecting outside of the normal WT θ range (~1.5 SD of the mean θ), whereas in mutant Disc1 mice there was a marked increase in the number of neurons with apical dendrites projecting outside of this range (~32% of tested young neurons). In addition, cells from mutant Disc1 mice displayed a wider range of orientation angles with no correlation between migration distance and θ (data not shown).

During maturation, granule cells experience a sequence of morphological rearrangements, starting with cell morphotypes typified by a single, well delineated primary dendrite and ending with a characteristic morphotype with multiple primary branches extending directly from the cell soma (23). In both mutant and WT mice, the vast majority of immature granule cells extended only one primary apical dendrite with no apparent genotypic differences in the fraction of morphotypes (data not shown). Analysis of their dendritic tree revealed a small, nonsignificant trend toward a decrease in apical dendritic branchpoints and dendritic length in mutant mice (P = 0.098) (Fig. S4D). In addition, no genotypic differences were observed in soma size (data not shown).

Finally, we examined whether the observed alterations in the spatial distribution and orientation are accompanied by alter-
corroborate this finding, we injected mutant Disc1 mice and their WT littermates. Note the presence of DCX-positive neurons in the deeper layer of the GCL (arrows) of mutant mice. The dotted line represents the outer edge of the GCL. (B) Quantification of neuronal distribution performed by using the χ² test in a contingency analysis. n = 54 cells were analyzed for each genotype. (C–E) Dendritic orientation of immature neurons. (C) Representative images of DCX-positive neurons from HOM mutant Disc1 mice and their WT littermates. (D and E) Quantification was performed by defining the angle of orientation (θ) for each apical dendrite (D) and calculating the mean change in θ (E). (F and G) Quantification of proliferating cells in the GCL. (F) Representative images of DCX-positive immature neurons (Upper) and BrdU-labeled neural precursor cells (Lower) in the DG of HOM mutant Disc1 mice as compared with their WT littermates. (G) Quantification of DCX-positive immature neurons (Left) and BrdU-labeled neural precursor cells (Right). Values represent mean ± SEM. * P < 0.05; ** P < 0.001. (Scale bars: A and F, 100 μm; C, 25 μm.)

Mature Granule Cells. We also analyzed GFP-expressing granule cells in mutant Disc1 mice crossed to the Thy1-GFP reporter strain. The Thy1 promoter directs expression in postmitotic neurons (24) and, as expected, the vast majority of GFP-expressing cells represent mature neurons that do not express DCX (Fig. 3A and B). Dendritic orientation of DG granule cells. (A) Representative images of GFP-labeled mature granule cells. (B) Quantification of dendritic misorientation. (C and D) Dendritic length of DG granule cells. (C) Representative tracings of GFP-labeled mature granule cells. (D) Quantification of total dendritic length plotted against their position in the GCL. WT/HOM (n): all (14/17); top (4/6); center (6/7); bottom (4/4). (E and F) Dendritic spine density of DG granule cells. Representative images of dendritic spines (E) and quantification of their numbers (F). Values represent mean ± SEM. * P < 0.05; ** P < 0.001; *** P < 0.0001. (Scale bars: A, 25 μm; C, 50 μm; E, 5 μm.)
A mutation in Disc1 leads to specific pattern of cognitive deficits. (A) Fear conditioning. (Left) Conditioned freezing during the cued test (n = 9 WT, 9 HOM). Percent of time freezing during the 60 s before tone presentation (pretone) and during the 20-s tone presentation (tone). (Right) Percent time freezing during the 4 min of the contextual test. (B) Novel object recognition. Although the ability to recognize a familiar object significantly decreased with time, there were no differences between genotypes (n = 9 WT, 9 HOM). (C) Morris water maze. (Left) Latency to reach a visible platform did not differ between genotypes (n = 9 WT, 9 HOM). (Center and Right) Both genotypes spent more time in the target quadrant during the probe trials. (D) Win-shift task. Both genotypes made comparable numbers of within-phase (Left) and across-phase (right) errors (n = 9 WT, 9 HOM). (E) Two-choice DNMP task. Both HET and HOM mutant Disc1 mice made significantly more errors during training (Left) and testing (Right; n = 10 WT, 11 HET, 11 HOM). Values represent mean ± SEM. n.s., not significant; *, P < 0.05.
nents of WM (see SI Text). There was a main effect of training day indicating learning across days \( [F(4, 116) = 4.6, P = 0.0018] \) and also a main effect of genotype \( [F(2, 29) = 4.2, P = 0.024] \). Both HOM and HET mutant Disc1 mice showed a clear learning deficit compared with WT littermate controls and made more errors across training days (Fig. 5E; Dunnett’s posthoc test, \( P < 0.05 \)). Longer delays randomly introduced between the sample and choice phases increased errors \( [F(2, 58) = 11.2, P < 0.0001] \), and again a main effect of genotype was found \( [F(2, 29) = 4.8, P = 0.015] \) (Fig. 5E Right). Planned contrasts showed that both HOM and HET mutant mice made more errors than WT (Student’s \( t \) test, \( P < 0.05 \)) with no difference between each other (Student’s \( t \) test, \( P = 0.18 \)). These results are not caused by nonspecific motivational or motor effects because response times and average trial completion times did not differ between genotypes (see SI Text and Fig. S6).

Discussion

One finding of this study is the strong regional selectivity of the observed morphological alterations in HPC and mPFC. This pattern could be related to the intrinsic properties of neurons or a cell type-specific contribution of Disc1 during embryonic and postnatal development dictated, at least in part, by its expression pattern (15). The mechanism behind the cellular aberrations may be related to the putative role of Disc1 at the centrosome (7) or in controlling cAMP levels (5, 27–29), although we cannot exclude other, yet-unexplored pathways. Morphological disturbances consistent with dendritic misorientation or impaired dendritic growth have been described in schizophrenia, albeit inconsistently (30, 31), and a recent study provided preliminary evidence for impaired adult neurogenesis in individuals with schizophrenia (32).

Kamiya et al. (6) have shown that shRNA-mediated depletion of Disc1 in embryonic cortical neurons inhibited their migration and induced misorientation and shortening of primary dendrites. Our analysis failed to confirm a widespread inhibition of migration in the DG, but rather revealed evidence consistent with “enhanced” migration, perhaps reflecting decreased sensitivity to repulsive guidance cues. This discrepancy could be caused by the different methodologies used, but it could also represent two different facets of misinterpretation of positional cues caused by impaired Disc1 function (33). In addition, in our model, both the dendritic misorientation and impaired growth phenotypes were restricted to DG neurons and not found in pyramidal cortical neurons. Although the reason for this difference is unclear, it is possible that such morphological deficits are of transient nature in some populations of neurons while they persist in others.

A more recent study using acute shRNA-mediated down-regulation of Disc1 in newly born DG cells has also independently reported mispositioning of young neurons in the GCL (34). However, unlike our study, in that study this phenotype was accompanied by enhanced dendritic growth and accelerated spine formation, which led the authors to speculate that Disc1 down-regulation results in accelerated functional neuronal integration. Instead, our results suggest that the newly generated DG neurons in mutant Disc1 mice may be actually compromised and possibly not able to integrate into DG functional circuits. This possibility appears to be supported by the additional observation that mature granule cells show impaired dendritic growth and reduced number of spines. These findings raise an important general issue pertaining to the information obtained by models based on shRNA-mediated approaches as opposed to models based on germ-line genetic lesions. They also highlight important differences between these approaches that have to do with the timing and magnitude of the genetic disruption and the unique induction of compensatory responses that could be activated in germ-line genetic lesions to buffer against developmental insults.

The other finding of our study is the pattern of the observed cognitive deficits. Cognitive analysis (the most comprehensive of a Disc1 mouse model reported to date) assessing various forms of memory revealed a significant deficit in a WM task that depends on the robust and active maintenance of information within WM networks in the face of irrelevant and competing information (35). This result is consistent with our previous findings with the same mutant Disc1 mice using another WM task (2) and two subsequent studies showing spatial WM deficits in other Disc1 models (9, 10). Lack of deficits in HPC-dependent cognitive assays of reference, recognition, and associative memory are consistent with the observed normal synaptic transmission and long-term plasticity at CA1/CA3 synapses. However, given the rapidly expanding study of the DG role in cognition (36–38), future studies using additional behavioral paradigms and larger experimental groups may reveal that Disc1 deficiency affects restricted aspects of HPC-dependent cognition. In fact, although WM-dependent learning and performance critically depends on the functional integrity of the mPFC in rodents, manipulations of DG also produces spatial WM deficits in some cognitive paradigms (37), indicating that impaired DG and mPFC function may be interacting to contribute to the observed cognitive profile. Overall, Disc1 deficiency leads to robust and reliable deficits in WM tasks with high executive components, suggesting these deficits may relate to WM and executive dysfunction observed in psychosis (17).

Independent of the underlying mechanism, our results strongly suggest that a mutation of endogenous mouse Disc1 that resembles closely the one observed in an affected family has highly selective effects on brain structure and function. These effects likely represent genuine links between this well defined Disc1 risk allele and disease susceptibility.

Materials and Methods

Animals. Genetically engineered mutant Disc1 mice contain the same Disc1 mutation backcrossed in C57BL/6J, as described by Koike et al. (2). All analyses were performed with 2- to 5-month-old male littermates produced by mating of HET mice. All animal procedures were approved by the Columbia University Institutional Animal Care and Use Committee.

Analysis of Migration, Dendritic Orientation, and Complexity. Analysis was essentially done as described (20, 22). For the migration analysis (20), confocal images of the DG were taken, and for each image the distance between the furthest migrating neuron and the SGZ/hilus border was measured. The furthest migrating cell was assigned to one of six 10-μm bins along the vertical axis of the DG. To analyze the apical dendrite orientation, ImageJ software was used to define a line from the center of the soma through 50–75 μm of the dendrite. The angle (θ) by this line relative to a line from the soma perpendicular to the SGZ was expressed in degrees (22). The quantification of dendritic complexity of granule cells and pyramidal neurons was performed essentially as described (18). Detailed descriptions of the methods can be found in SI Text.

BrdU Labeling, Immunohistochemistry, and Quantification of BrdU- and DCX-Positive Cells. Analysis was performed as described (39). Mice were injected with BrdU (50 mg/kg) for 12 consecutive days. BrdU and DCX stainings were performed on every sixth section of the entire HPC.

Western Blotting. Whole brains from postnatal day 2 pups or hippocampi from adult mice were dissected out, homogenized, and analyzed by Western blotting. The following antibodies were used: purified N-terminal anti-Disc1 Ab (1:100), purified C-terminal anti-Disc1 Ab (1:100), anti-Disc1 Ab from Santa Cruz Biotechnology (N16; 1:500) and anti-Disc1 Ab from Zymed (ZMD.488; 1:500). Conditions used for immunohistochemistry are described in SI Text.

Behavioral Assays. Behavioral studies were carried out as described (38, 40–42) (see also SI Text) in three groups of male mice (2–5 months old). Group 1 underwent training in the Morris water maze followed by the win-shift version of the radial arm maze and the novel object recognition test. Group 2
was subjected to fear conditioning test. Group 3 performed the two-choice DNMP task.

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