Disc1 is mutated in the 129S6/SvEv strain and modulates working memory in mice

Hiroko Koike*†‡, P. Alexander Arguello‡§, Mirna Kvajo*†, Maria Karayiorgou*¶, and Joseph A. Gogos†§¶

*Human Neurogenetics Laboratory, The Rockefeller University, 1230 York Avenue, New York, NY 10021; †Department of Physiology and Cellular Biophysics, Columbia University Medical Center, 630 West 168th Street, New York, NY 10032; and §Center for Neurobiology and Behavior, Columbia University Medical Center, 701 West 168th Street, New York, NY 10032

Communicated by David E. Housman, Massachusetts Institute of Technology, Cambridge, MA, December 26, 2005 (received for review December 15, 2005)

Disrupted-In-Schizophrenia (*DISC1*) is a leading candidate schizophrenia susceptibility gene. Here, we describe a deletion variant in *mDisc1* specific to the 129S6/SvEv strain that introduces a termination codon at exon 7, abolishes production of the full-length protein, and impairs working memory performance when transferred to the C57BL/6J genetic background. Our findings provide insights into how *DISC1* variation contributes to schizophrenia susceptibility in humans and the behavioral divergence between 129S6/SvEv and C57BL/6J mouse strains and have implications for modeling psychiatric diseases in mice.

animal model | gene mutation | psychiatric disorder | schizophrenia

Schizophrenia is a common and genetically complex psychiatric disorder. Several leading candidate susceptibility genes have been identified (1), including *DISC1*. The position of this gene was initially pinpointed by a balanced translocation (1;11)(q42.1;q14.3) that was strongly linked to major neuropsychiatric diseases, including schizophrenia, depression, and bipolar disorder in a large Scottish family. The 1q breakpoint involves two genes, *DISC1* and *DISC2* (a noncoding, presumably regulatory RNA) (2) and truncates *DISC1* immediately after exon 8. More recent large-scale linkage and follow-up association studies in families from Finland identified *DISC1* as a positional candidate gene from the 1q42 locus (3). In addition, several polymorphisms in *DISC1* were reported to be associated with schizophrenia-related cognitive endophenotypes (4–6). Although the function of *DISC1* is poorly understood, the gene is expressed the highest during early development, is associated with numerous cytoskeletal proteins, and could be involved in cell migration and neurite outgrowth and could play a role in centrosomal, microtubule and mitochondrial function, as well as in phosphodiesterase signaling (7–9).

Results

In the process of a gene targeting experiment at the *mDisc1* locus (see *Supporting Methods*, which is published as supporting information on the PNAS web site), we identified a 25-bp deletion in exon 6 of the 129S6/SvEv *mDisc1* allele. We used RT-PCR of mouse brain RNA from 129S6/SvEv (Taconic Farms) and C57BL/6J (The Jackson Laboratory) strains to examine sequences surrounding all *mDisc1* exons. We confirmed the 25-bp deletion in exon 6 of 129S6/SvEv *mDisc1* transcript. The deletion was absent in the C57BL/6J strain (Fig. 1 *a* and *b*). We also confirmed by direct sequencing the presence of this deletion in genomic DNA isolated from tails of Taconic 129S6/SvEv mice, as well as from ES cells (data not shown). We therefore conclude that this deletion represents a natural 129S6/SvEv-specific polymorphism. As a control, we amplified exon 6 from genomic DNA from five other inbred strains (BALB/cJ, CBA/J, C3H/HeJ, DBA/2J, and AKR/J strains). We did not detect this deletion variant in any strain (Fig. 1*c*), although we found that BALB/cJ and C3H/HeJ strains harbor a single, nonconservative (Glu529His) nucleotide polymorphism within the same 25-bp region. The identified 25-bp deletion induces a frameshift in the reading frame, resulting in 13 novel amino acids, followed by a premature stop codon in exon 7 (Fig. 1*b*). Both human and mouse genes present a relatively complex pattern of alternative

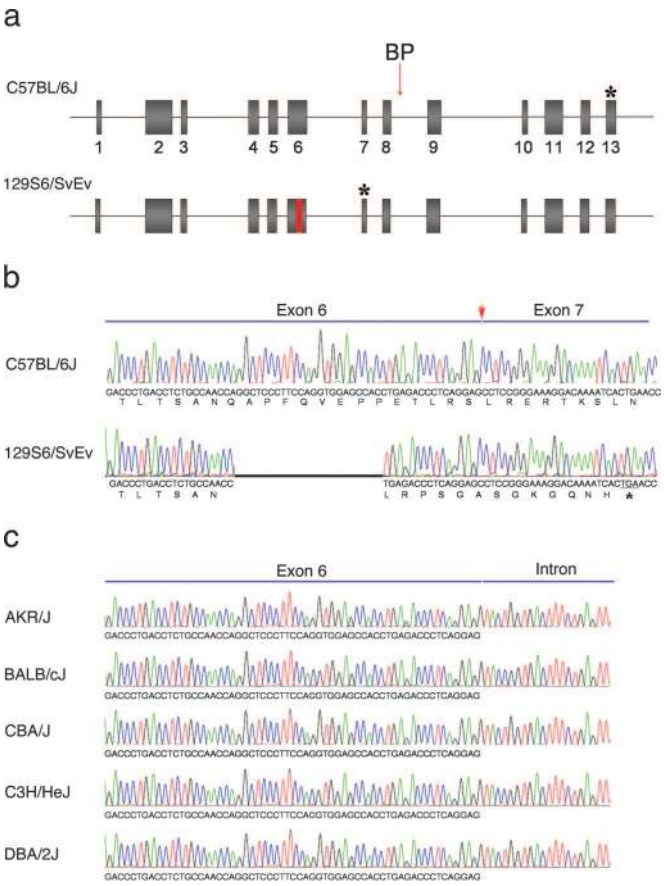

Fig. 1. *mDisc1* deletion variant in the 129S6/SvEv strain. (*a*) Genomic structure of mDisc1 gene. The position of the 129S6/SvEv deletion polymorphism in exon 6 is indicated by a red bar. BP, breakpoint in the Scottish family; *, premature stop codon. (*b*) Chromatograms from sequencing *mDisc1* exon 6 in C57BL/6J and 129S6/SvEv mouse strains. The 129S6/SvEv 25-bp deletion variant in exon 6 (black horizontal line) and the introduced premature termination codon (*) at exon 7 are indicated. Exon 6 was amplified from brain cDNA for the 129S6/SvEv and C57BL/6J strains although similar results were obtained from genomic PCR amplification (data not shown). Red arrowhead indicates the splice junction between exons 6 and 7. (*c*) Chromatograms from sequencing *mDisc1* exon 6 in five other mouse strains. Indicated is genomic sequence from *mDisc1* exon 6 and surrounding intron 6 in AKR/J, BALB/cJ, CBA/J, C3H/HeJ, and DBA/2J strains.

Conflict of interest statement: No conflicts declared.

‡H.K. and P.A.A. contributed equally to this work.

¶To whom correspondence may be addressed. E-mail: karayim@rockefeller.edu or jag90@columbia.edu.

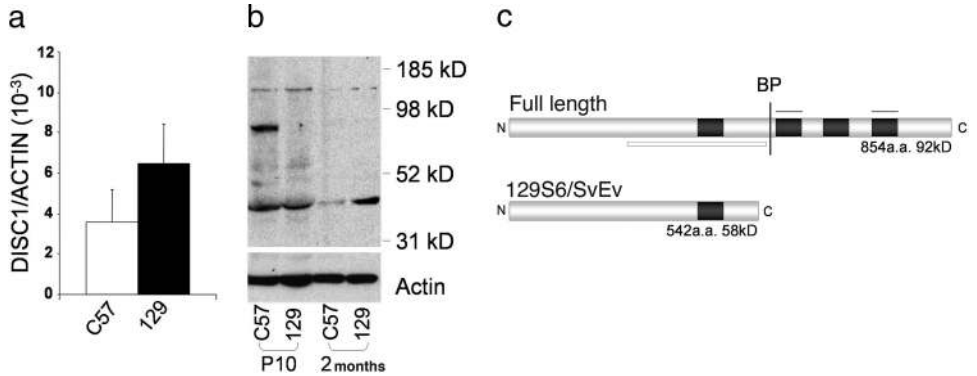

Fig. 2. mDisc1 protein levels in the 129S6/SvEv strain. (*a*) Stability and abundance of the full-length transcript in the 129S6/SvEv strain. Transcript levels were estimated by quantitative RT-PCR on total RNA isolated from P10 mouse brain, by using a C-terminal probe. No internal promoter usage has been described at this locus, and therefore this method provides a good indication of the stability and abundance of the full-length transcript in the 129S6/SvEv strain. C57, C57BL/6J; 129, 129S6/SvEv. (*b*) The effect of the 129S6/SvEv exon 6 variant on protein expression levels. Shown is Western blot analysis with an antibody raised against the N-terminal region of mDisc1. Three major bands are observed. The ≈96-kDa band most likely corresponds to the predicted 92-kDa full-length protein. The ≈45-kDa signal may correspond to a previously described short form variant (NCBI database: NM_170596) that includes exons 1–3. The ≈150-kDa signal seems independent of the full length protein and may represent a highly stable complex (27) involving shorter Disc1 variant(s) or a nonspecific signal. C57, C57BL/6J; 129, 129S6/SvEv. (*c*) Predicted protein domains of the mDISC1 protein from C57BL/6J and 129S6/SvEv strains. Black boxes indicate predicted coiled-coil domains identified by COILS search (www.ch.embnet.org/software/COILS_form.html). Upper horizontal lines indicate the predicted leucine zipper domain. Open bar indicates the approximate position of the peptide antigen. BP, approximate position of the truncation in the human orthologue induced by the breakpoint in the Scottish family.

splicing (10). Nevertheless, RT-PCR amplification (using several sets of primers flanking exon 6) and sequencing of 129S6/SvEv mouse brain cDNA confirmed that every splice variant detected retains exon 6 (data not shown). Because of the low *mDisc1* transcript abundance (10), we used quantitative RT-PCR of brain RNA to estimate full-length transcript levels (Fig. 2*a*). We did not detect any decrease in transcript abundance in 129S6/SvEv relative to the "wild-type" C57BL/6J strain (Fig. 2*a*), suggesting that the mutated transcript escapes nonsense-mediated decay (11).

To investigate the effect of the 129S6/SvEv exon 6 deletion variant on protein expression levels, we raised a polyclonal antibody against the N-terminal portion of the protein (amino acids 272–537) and performed Western blot analysis in total brain protein extracts from both C57BL/6J and 129S6/SvEv strains. We identified several major bands of ≈150 kDa, 96 kDa, and 45 kDa in lysates from early postnatal C57BL/6J mice (Fig. 2*b*). Consistent with previous studies (12, 13), levels of Disc1 seem to be higher during early postnatal neurodevelopment compared with the adult brain, although the exact patterns of protein distribution may differ among studies according to the specificity and avidity of the used antisera. Specifically, the ≈96-kDa band, which very likely corresponds to the predicted 92-kDa full-length protein (Fig. 2*c*), seems to be developmentally regulated and decreases in intensity in lysates prepared from adult brains. The same band is undetectable in lysates prepared from either early postnatal or adult 129S6/SvEv brains. The predicted C-terminally truncated protein product (58 kDa) (Fig. 2*c*) is also not detectable in these lysates. Considering that the full-length *mDisc1* transcript can be detected in 129S6/SvEv mice, these data likely indicate that the truncated protein missing the C-terminal portion is relatively unstable.

To facilitate analysis of the effects of *Disc1* deficiency on mouse behavior and cognition, we transferred a modified 129S6/SvEv *mDisc1* allele (see *Supporting Methods* and Fig. 4, which is published as supporting information on the PNAS web site) onto a C57BL/6J genetic background, employing a marker-assisted selection congenic strategy. The resultant *mDisc1*-deficient strain retained >98% of the C57BL/6J genome after five generations of backcrossing. As expected, the full-length Disc1 protein was absent in total brain lysates from homozygous mice (data not shown), as well as in lysates from purified brain mitochondria, where the protein is thought to be localized (7) (see Fig. 4). Gross brain morphology appeared normal in homozygous mutant mice by initial low-resolution histological examination of sections (Fig. 3*a*).

Recent association studies in patients with schizophrenia and their unaffected siblings suggest that *DISC1* variants might modulate visuospatial working memory, a prefrontal-based endophenotypic trait previously observed to co-vary with genetic liability to schizophrenia (4, 5). The effect, however, of the implicated *DISC1* variants on the function or expression of the gene is unknown. We used the *mDisc1*-deficient C57BL/6J mice to investigate whether the potential contribution of DISC1 on prefrontal-based cognitive performance is conserved across species. Specifically, we asked whether *mDisc1* deficiency in mice affects spatial working memory performance as assessed in a delayed non-match to place task. The task contingencies used in the present study are designed to engage frontal regions of the rodent neocortex by using retention intervals within the working memory range (5–30 s) and minimizing the intertrial interval to allow for proactive interference, which taxes prefrontal cortical resources (14). Using these task parameters, the integrity of the frontal cortex is critical (15) but the contribution of fronto-hippocampal circuitry in similar tasks may also indicate a role for the hippocampus (16).

C57BL/6J mice deficient in *mDisc1* learned the task and performed as well as WT littermates during training. In the working memory test, however, *mDisc1*-deficient mice showed a consistent impairment compared with WT littermate controls ($F_{2,25} = 5.29$, $P = 0.01$; Fig. 3*b*) with no evidence for a genotype × sex effect ($F_{2,22} = 0.16$, $P = 0.85$). This impairment was observed in both heterozygous and homozygous mutant mice, suggesting that the gene is haploinsufficient in relation to this phenotype. This gene-dosage dependent effect of *DISC1* on the functional integrity of the prefrontal cortex may contribute to the increased schizophrenia risk in heterozygous carriers of the (1;11)(q42.1;q14.3) translocation. There were no differences among genotypes in the time taken to complete the test ($F_{2,25} = 0.11$, $P = 0.89$, Fig. 3*b*), ruling out any nonspecific motoric or motivational effects. Moreover, in control experiments, we did not detect any significant changes in indices of locomotor activity

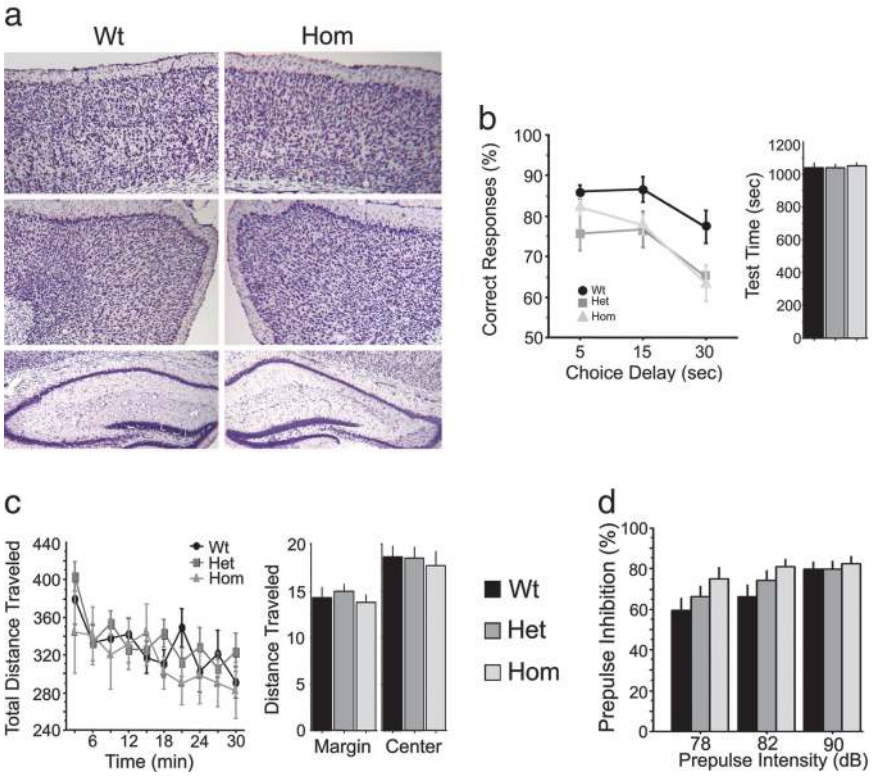

Fig. 3. *mDisc1* deficiency results in impaired working memory performance. (*a*) Gross brain morphology in *mDisc1*-deficient mice. Shown are cresyl violet-stained coronal sections through the brain of adult *mDisc1*-deficient mice (Hom) and their WT littermates (Wt). (*Top*) Prefrontal cortex at ≈−1.70 mm Bregma. (*Middle*) Cortex at ≈−2.18 mm Bregma. (*Bottom*) Hippocampus. (*b*) Working memory performance in a delayed non-match to place task. (*Left*) Mean percentage of correct responses (± SEM) for WT (Wt), heterozygous (Het), and homozygous (Hom) mice. Note that vertical axis starts at 50% correct responses, which represents baseline response accuracy expected by chance. (*Right*) Mean time (± SEM) to complete the working memory task measured in seconds. There were no differences between genotypes either in the number of days to reach criterion or in the level of performance in the 3 days before testing. (*c*) Spontaneous locomotion. (*Left*) Total distance traveled (± SEM) as a function of time in 3-min bins. (*Right*) Distance traveled (± SEM) over a 30-min period in the margins and center of a novel open field environment. (*d*) Prepulse inhibition. Percentage inhibition (± SEM) at different levels of prepulse intensities. There were no observed differences in background levels or startle responses.

(Fig. 3*c*) nor any genotype-based impairment of prepulse inhibition [indeed, a trend toward higher inhibition in homozygous mice was evident ($F_{2,25} = 2.46$, $P = 0.10$; Fig. 3*d*)].

Discussion

Here, we describe a deletion variant in *mDisc1* specific to the 129S6/SvEv strain that introduces a termination codon at exon 7 and abolishes production of the full-length protein. To facilitate analysis of the effects of *Disc1* deficiency on mouse behavior and cognition, we transferred a modified 129S6/SvEv *mDisc1* allele onto a C57BL/6J genetic background employing a marker-assisted selection congenic strategy. The resultant *mDisc1*-deficient strain retained >98% of the C57BL/6J genome and demonstrated a specific deficit in working memory performance. Our experimental design decreases the probability of contribution in trans of unrelated background genes but does not address the well known "flanking gene problem" (17), which can complicate interpretation of all studies in which genes are "knocked-out" by means of homologous recombination. Even after backcrossing the mutant *Disc1* allele to the C57BL/6J strain, the resulting congenic line will still harbor a part of chromosome 8 from the 129S6/SvEv donor strain that contains a number of flanking genes. Variants of these genes on their own or by interacting with *Disc1* could in principle contribute to the observed behavioral performance of the *Disc1*-deficient strain. Backcrossing to 129S6/SvEv genetic background (17) is not an option in this case, and homologous recombination in ES cells from C57BL/6J (18, 19) represents possibly the best way to address this issue in future experiments.

Cognitive impairment has always been regarded as a hallmark feature of schizophrenia. Recently, cognitive dysfunction has been recognized as an enduring core deficit that serves as a strong indicator of specific genetic liability to the disease and a primary target for pharmacotherapy (20, 21). Individuals with schizophrenia show varying degrees of deficiency in a diverse range of cognitive domains subserved by frontostriatal, hippocampal, and parietal regions (such as working memory, short-term and episodic memory, attention, executive functions, and learning) (20). In particular, working memory deficits that are attributed primarily to prefrontal dysfunction are considered a cardinal cognitive symptom of schizophrenia (22). The convergence between behavioral analysis presented here and recent correlative genetic studies in humans supports the notion that deficits in spatial working memory performance are a good indicator of genetic liability to schizophrenia and suggests that impaired function of the *DISC1* gene may influence schizophrenia risk, phenotypic expression, or both, in part by affecting the functional integrity of the prefrontal cortex. The effect of *DISC1* deficiency, however, may not be exclusive to the frontal cortex. Further analysis of *mDisc1*-deficient mice will address whether other schizophrenia-related cognitive domains subserved by different anatomical regions are affected as well.

There are two additional implications of our findings. First, our results suggest that the absence of full-length Disc1 protein in 129S6/SvEv may underscore at least some of the differences in cognitive traits that characterize this strain of mice and distinguish it from the C57BL/6J strain (23). Coding variation that results in drastic changes in protein structure (such as premature termina-

tion) is expected to play an important role in behavioral divergence between mouse strains, and preliminary estimates predict >100 premature termination codons in genes of the 129 strains compared with C57BL/6J (24). Second, our results have implications for modeling psychiatric disorders in mice. Given that the genetic component of schizophrenia and other major psychiatric disorders involves interaction among several genes (25), our finding suggests that analysis of targeted orthologs of susceptibility genes in the 129S6/SvEv background, already compromised in one schizophrenia-related genetic pathway, could increase the penetrance of introduced mutations and reveal features masked in the traditionally used C57BL/6J background.

Materials and Methods

Protein Extraction and Western Blot Analysis. Total brain tissues from 5-, 10-, and 60-day-old mice were homogenized in 25 mM Tris, 125 mM sodium chloride, 1% Triton X-100, and Complete Proteinase Inhibitor (Roche Applied Science). Samples were adjusted to 7 μg of total protein. Western blotting was performed by using 4–12% gradient NuPage gels (Invitrogen) and purified serum IgG from rabbits injected with the purified N-terminal (amino acids 272–537) mDisc1 peptide.

Quantitative Real-Time RT-PCR. mRNA expression levels were quantified by using real-time RT-PCR analysis on an ABI Prism 7900 sequence-detection system (PE Applied Biosystems). The quantitative real-time PCR was performed in a volume of 25 μl with 500 ng of cDNA in the universal master mix (Qiagen, Valencia, CA), 400–900 nM primers (Sigma Genosys), and 200 nM fluorogenic internal probe (Applied Biosystems). Samples were heated for 2 min at 50°C and for 10 min at 95°C and were amplified for 40 cycles of 15 s at 95°C and 1 min at 60°C. A serial dilution of cDNA derived from mouse brain total RNA (BD Biosciences) was amplified in parallel as a control to verify amplification efficiency within each experiment. Relative mRNA expression levels of the gene of interest for each sample were directly normalized for input RNA against β-actin expression. All experiments were performed in triplicate. Data are given as mean ± SEM, and the comparisons between the two experimental groups are made by standard Student's t tests. Primer sequences are as follows: TaqMan probe, 6FAM-ACGCCGCCCACAAAACACGC-MGBNFQ; forward primer, 5′-GAACAGCAGAAGGCTGGGC-3′; reverse primer, 5′-GACCTTCCAACACTTCCATGC-3′). The analysis was done by Applied Biosystems SDS 2.2 software.

Primary Antibody. Rabbit polyclonal antibody was raised against a purified N-terminal region of mDisc1 (amino acids 272–537) that was fused to GST and expressed in bacteria, by using standard procedures. The antiserum was affinity purified, and its specificity was determined by Western blot analysis on bacterial extracts, by using the preimmune serum as a control.

Behavioral Analysis. To assess working memory, a delayed nonmatch to place task was used as described (15). Briefly, 6- to 8-week-old WT (n = 10), heterozygous (n = 10), and homozygous (n = 9) mice, with approximately equal male/female representation, were food-restricted to 80% of their free body weight, followed by 3 days of habituation to the maze. Then, 2 days of forced alternation followed in which only the left or right arm was open, followed by the opposite arm. On each subsequent day of training, a pseudorandom sequence of 10 left or right turns was chosen (e.g., LRLLRLRRLR). The mice were given a forced run (the sample) to one arm and then, after a 5-s delay, given access to the original and opposite arm (the non-match) with an intertrial interval of 40 s. Correct choices were reinforced with food reward at the end of the correct arm. After mice made at least 70% correct choices on 3 consecutive days, the testing phase began. In this case, delays of 5, 15, or 30 s were randomly introduced between the sample run and choice run. Mice were given 4 trials of each delay on 3 days of testing for a total of 12 trials for each delay.

Spontaneous locomotion and sensorimotor gating were assessed as described (25) in WT (n = 13), heterozygous (n = 14), and homozygous (n = 9) mice. For locomotion, data were collected every minute for 30 min, and 10 measures were analyzed, including distance traveled in the margin and center of the open field, vertical and horizontal repetitive movements, and the number of rearings. For sensorimotor gating, prepulse inhibition was assessed with prepulse intensities of 78, 82, and 90 dB. All data were analyzed with repeated measures or one-way ANOVA with genotype as the factor.

Histological Analysis. Mice were transcardially perfused with ice-cold PBS and 4% paraformaldehyde (PFA). Brains were rapidly dissected out, equilibrated in sucrose, and quickly frozen in Tissue-Tek O.C.T (Sakura Finetek, Torrance, CA). Cryostat (20 mm) sections were air-dried and stained with cresyl violet by using a modified method described in ref. 26.

Mitochondria Isolation. Prefrontal cortex and hippocampus from P6 *mDisc1*-deficient1 mice and their WT littermates were rapidly dissected out and homogenized in ice-cold PBS using a Dounce homogenizer. Mitochondria were isolated by using the Mitochondria Isolation Kit for Tissue from Pierce, according to the instructions of the manufacturer. The mitochondrial pellet was boiled in loading buffer, and Western blotting was performed by using 4–12% gradient NuPage gels (Invitrogen) and purified serum IgG from rabbits injected with the purified N-terminal (amino acids 272–537) mDisc1 peptide (1:1,000). Prohibitin was detected with rabbit anti-prohibitin (1:2,000; Abcam, Cambridge, MA).

We thank C. Frazier and A. Garcia-Williams for technical support and assistance with the mouse colony and Ruby Hsu for help in the generation of the targeting construct. This research was supported in part by National Institutes of Health Grant MH67068 and the New York Academy of Sciences (J.A.G.). H.K. is supported by a National Alliance for Research on Schizophrenia and Depression (NARSAD) Young Investigator award, and P.A.A. is supported by a Society for Neuroscience predoctoral fellowship. J.A.G. is also the recipient of a McKnight Brain Disorders Award, is an EJLB Scholar, and is a Vicente NARSAD Young Investigator.

1. Gogos, J. A. & Gerber D. J. (2006) *Trends Pharmacol. Sci*., in press.
2. Millar, J. K., Wilson-Annan, J. C., Anderson, S., Christie, S., Taylor, M. S., Semple, C. A., Devon, R. S., Clair, D. M., Muir, W. J., Blackwood, D. H., *et al*. (2000) *Hum. Mol. Genet*. **9,** 1415–1423.
3. Ekelund, J., Hennah, W., Hiekkalinna, T., Parker, A., Meyer, J., Lonnqvist, J. & Peltonen, L. (2004) *Mol. Psychiatry* **9,** 1037–1041.
4. Hennah, W., Tuulio-Henriksson, A., Paunio, T., Ekelund, J., Varilo, T., Partonen, T., Cannon, T. D., Lonnqvist, J. & Peltonen, L. (2005) *Mol. Psychiatry* **10,** 1097–1103.
5. Callicott, J. H., Straub, R. E., Pezawas, L., Egan, M. F., Mattay, V. S., Hariri, A. R., Verchinski, B. A., Meyer-Lindenberg, A., Balkissoon, R., Kolachana, B., *et al*. (2005) *Proc. Natl. Acad. Sci. USA* **102,** 8627–8632.
6. Cannon, T. D., Hennah, W., van Erp, T. G., Thompson, P. M., Lonnqvist, J., Huttunen, M., Gasperoni, T., Tuulio-Henriksson, A., Pirkola, T., Toga, A. W., *et al*. (2005) *Arch. Gen. Psychiatry* **62,** 1205–1213.
7. Millar, J. K., James, R., Christie, S. & Porteous, D. J. (2005) *Mol. Cell. Neurosci*. **30,** 477–484.
8. Millar, J. K., Pickard, B. S., Mackie, S., James, R., Christie, S., Buchanan, S. R., Malloy, M. P., Chubb, J. E., Huston, E., Baillie, G. S., *et al*. (2005) *Science* **310,** 1187–1191.
9. Kamiya, A., Kubo, K. I., Tomoda, T., Takaki, M., Youn, R., Ozeki, Y., Sawamura, N., Park, U., Kudo, C., Okawa, M., *et al*. (2005) *Nat. Cell Biol*. **7,** 1067–1078.
10. Ma, L., Liu, Y., Ky, B., Shughrue, P. J., Austin, C. P. & Morris, J. A. (2002) *Genomics* **80,** 662–672.

11. Holbrook, J. A., Neu-Yilik, G., Hentze, M. W. & Kulozik, A. (2004) *Nat. Genet.* **36,** 801–808.
12. Ozeki, Y., Tomoda, T., Kleiderlein, J., Kamiya, A., Bord, L., Fujii, K., Okawa, M., Yamada, N., Hatten, M. E., Snyder, S. H., *et al.* (2003) *Proc. Natl. Acad. Sci. USA* **100**, 289–294, and erratum (2004) **101,** 13969.
13. Schurov, I. L., Handford, E. J., Brandon, N. J. & Whiting, P. J. (2004) *Mol. Psychiatry* **9,** 1100–1110.
14. Baddeley, A. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 13468–13472.
15. Aultman, J. M. & Moghaddam, B. (2001) *Psychopharmacology* **153,** 353–364.
16. Jones, M. W. & Wilson, M. A. (2005) *PLoS Biol.* **3,** e402.
17. Gerlai, R. (1996) *Trends Neurosci.* **19,** 177–181.
18. Auerbach, W., Dunmore, J. H., Fairchild-Huntress, V., Fang, Q., Auerbach, A. B., Huszar, D. & Joyner, A. L. (2000) *BioTechniques* **29,** 1024–1032.
19. Schuster-Gossler, K., Lee, A. W., Lerner, C. P., Parker, H. J., Dyer, V. W., Scott, V. E., Gossler, A. & Conover, J. C. (2001) *BioTechniques* **31,** 1022–1026.
20. Bowie, C. R. & Harvey, P. D. (2005) *Psychiatr. Clin. N. Am.* **28,** 613–633.
21. Green, M. F., Nuechterlein, K. H., Gold, J. M., Barch, D. M., Cohen, J., Essock, S., Fenton, W. S., Frese, F., Goldberg, T. E., Heaton, R. K., *et al.* (2004) *Biol. Psychiatry* **56,** 301–307.
22. Goldman-Rakic, P. S. (1994) *J. Neuropsychiatry Clin. Neurosci.* **6,** 348–357.
23. Crawley, J. N., Belknap, J. K., Collins, A., Crabbe, J. C., Frankel, W., Henderson, N., Hitzemann, R. J., Maxson, S. C., Miner, L. L., Silva, A. J., *et al.* (1997) *Psychopharmacology* **132,** 107–124.
24. Adams, D. J., Dermitzakis, E. T., Cox, T., Smith, J., Davies, R., Banerjee, R., Bonfield, J., Mullikin, J. C., Chung, Y. J., Rogers, J., *et al.* (2005) *Nat. Genet.* **37,** 532–536.
25. Paterlini, M., Zakharenko, S. S., Lai, W. S., Qin, J., Zhang, H., Mukai, J., Westphal, K. G., Olivier, B., Sulzer, D., Pavlidis, P., *et al.* (2005) *Nat. Neurosci.* **8,** 1586–1594.
26. Simmons, D. M. & Swanson, L. W. (1993) *The Nissl Stain*, Neuroscience Protocols, Wouterlood, F. G., ed. (Elsevier Science, Amsterdam), 93-050-12, pp. 01–07.
27. James, R., Adams, R. R., Christie, S., Buchanan, S. R., Porteous, D. J. & Millar, J. K. (2004) *Mol. Cell. Neurosci.* **26,** 112–122.