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Behavioral Phenotypes of *Disc1* Missense Mutations in Mice

Steven J. Clapcote,[1,*] Tatiana V. Lipina,[1] J. Kirsty Millar,[2] Shaun Mackie,[2] Sheila Christie,[2] Fumiaki Ogawa,[2] Jason P. Lerch,[3] Keith Trimble,[3] Masashi Uchiyama,[4] Yoshiyuki Sakuraba,[4] Hideki Kaneda,[4] Toshihiko Shiroishi,[4] Miles D. Houslay,[5] R. Mark Henkelman,[3,6] John G. Sled,[3,6] Yoichi Gondo,[4] David J. Porteous,[2,*] and John C. Roder[1,6]

[1] Samuel Lunenfeld Research Institute at Mount Sinai Hospital, Toronto, ON M5G 1X5, Canada
[2] Medical Genetics Section, University of Edinburgh Centre for Molecular Medicine, Edinburgh EH4 2XU, United Kingdom
[3] Hospital for Sick Children, Mouse Imaging Centre and Auditory Science Laboratory, Toronto, ON M5G 1X8, Canada
[4] RIKEN Genomic Sciences Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan
[5] Molecular Pharmacology Group, Division of Biochemistry & Molecular Biology, IBLS, Wolfson Building, University of Glasgow, Glasgow G12 8QQ, United Kingdom
[6] University of Toronto, Departments of Medical Biophysics and Molecular & Medical Genetics, Toronto, ON M5S 1A1, Canada
*Correspondence: mail.steve@btinternet.com (S.J.C.), david.porteous@ed.ac.uk (D.J.P.)
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SUMMARY

To support the role of *DISC1* in human psychiatric disorders, we identified and analyzed two independently derived ENU-induced mutations in Exon 2 of mouse *Disc1*. Mice with mutation Q31L showed depressive-like behavior with deficits in the forced swim test and other measures that were reversed by the antidepressant bupropion, but not by rolipram, a phosphodiesterase-4 (PDE4) inhibitor. In contrast, L100P mutant mice exhibited schizophrenic-like behavior, with profound deficits in prepulse inhibition and latent inhibition that were reversed by antipsychotic treatment. Both mutant DISC1 proteins exhibited reduced binding to the known DISC1 binding partner PDE4B. Q31L mutants had lower PDE4B activity, consistent with their resistance to rolipram, suggesting decreased PDE4 activity as a contributory factor in depression. This study demonstrates that *Disc1* missense mutations in mice give rise to phenotypes related to depression and schizophrenia, thus supporting the role of *DISC1* in major mental illness.

INTRODUCTION

Schizophrenia is a common, chronic, and severely debilitating form of mental illness, typified by positive symptoms of hallucinations, delusions, and thought disorder; negative symptoms of decreased motivation, altered expressivity, and an inability to experience pleasure (anhedonia); selective cognitive deficits in attention, learning, and memory; and comorbid features of depression, substance abuse, and suicide (Ross et al., 2006; Lewis and Gonzalez-Burgos, 2006). It ranks as one of the leading causes of disability worldwide, but current treatments are at best palliative and will remain so until the molecular basis is better understood (Insel and Scolnick, 2006; Lewis and Gonzalez-Burgos, 2006). There is a strong genetic component to the illness. Indeed, over 130 genes have been reported to predispose to schizophrenia, but few have been replicated, and fewer still have biological support (Ross et al., 2006). One candidate is *Disrupted in schizophrenia 1* (*DISC1*), which was originally identified at one breakpoint of a chromosomal t(1;11) (q42.1;q14.3) translocation that cosegregates in a large Scottish family with major mental illness, including schizophrenia, bipolar disorder, and major depression (Millar et al., 2000; Blackwood et al., 2001).

Multiple independent linkage and association studies in diverse populations have since reported preliminary evidence in support of *DISC1* as a more generalized risk factor in schizophrenia and mood disorders (reviewed in Hennah et al. [2006], Porteous et al. [2006]). *DISC1* has also been reported to associate with impaired memory, reduced gray matter density and volume, and abnormal hippocampal volume and function (Callicott et al., 2005; Cannon et al., 2005; Hennah et al., 2005; Hashimoto et al., 2006). Although some of these associations have been related to known *DISC1* missense polymorphisms, to date, evidence for consistent allelotypic or genotypic association and robust associated dysfunction of DISC1 is lacking, and thus the criteria for causality have not been met.

A consistent pattern has emerged from DISC1 expression studies in the human, primate, and rodent brain, with evidence for developmental regulation and high protein levels in subregions, particularly the hippocampus, implicated in the pathogenesis of psychiatric disorders (Austin et al., 2003, 2004; Miyoshi et al., 2003; Ozeki et al., 2003; Schurov et al., 2004). DISC1 functions as a molecular scaffold, interacting with multiple proteins

required for neuronal migration, neurite outgrowth, signal transduction, cyclic adenosine monophosphate (cAMP) signaling, cytoskeletal modulation, and translational regulation (reviewed in Porteous et al. [2006], Ross et al. [2006], Camargo et al. [2007]). Among the known DISC1 binding partners, cAMP-hydrolyzing PDE4B is particularly noteworthy on four counts. First, the *PDE4B* gene was found to be disrupted by a translocation in two related individuals with psychosis (Millar et al., 2005). Second, PDE4 isoforms are targets of inhibition by rolipram (Wachtel, 1983) and linked through animal models to learning, memory, and mood (Arguello and Gogos, 2006; O'Donnell and Zhang, 2004). Third, PDE4B activity is dynamically regulated by DISC1 (Millar et al., 2005). Fourth, PDE4 isoforms are now well recognized as underpinning compartmentalized cAMP signaling (Di Benedetto et al., 2006) through being tethered to specific scaffold proteins such as β-arrestin and RACK1 (Houslay et al., 2005).

We have proposed that allelic variation of *DISC1* might have pleiotropic effects, depending on the location and type of sequence change, the selective effects these may have on specific protein interactions, and the consequential impact on the DISC1 pathway as this relates to normal and dysfunctional brain development, biochemistry, and behavior (Porteous et al., 2006). Further clinical studies can address some of the outstanding questions, but this approach is restricted by the limited scope for experimentation and access to appropriate tissue. We therefore sought to establish mutations in the mouse *Disc1* gene and then use cognate measures of behavior, brain anatomy, neurophysiology, and pharmacological response to support and extend the clinical findings. The method of choice was *N*-nitroso-*N*-ethylurea (ENU) mutagenesis, which induces point mutations at a high locus-specific rate (Coghill et al., 2002). ENU-induced mutations in *Disc1*, along with the recently discovered 25 bp deletion that arose spontaneously in the 129 strain (Clapcote and Roder, 2006; Koike et al., 2006), would form a series of mutant alleles through which to elucidate DISC1 function in the laboratory mouse. We report the differential impacts of two such mutations, one producing a depressive-like phenotype and the other resulting in a schizophrenic-like phenotype in mice with a C57BL/6J genetic background.

RESULTS

Identification of Missense Mutations in *Disc1*

DISC1 isoforms are encoded by 13 major exons, of which Exon 2 at 955 bp is the longest. Exon 2 is present in all known splice isoforms and encodes most of the protein head domain, which we have shown previously in the human to interact with PDE4B (Millar et al., 2005). Association between schizophrenia, schizoaffective disorder, and aspects of working memory in the human have been reported for Exon 2-spanning haplotypes (Cannon et al., 2005; Hennah et al., 2005; Maeda et al., 2006). We screened Exon 2 of *Disc1* in 1686 F1 progeny of ENU-mutagenized C57BL/6JJcl males and untreated DBA/2JJcl females, and de-

tected two independently-derived missense mutations. Mutant transcript *Disc1*^{Rgsc1393} has a 127A→T transversion (Figure 1B) resulting in amino acid exchange Q31L (Figure 1A), and mutant transcript *Disc1*^{Rgsc1390} has a 334T→C transition (Figure 1B) resulting in amino acid exchange L100P in the encoded protein (Figure 1A). The Exon 2 sequences of the C57BL/6JJcl and DBA/2JJcl parental strains are identical (data not shown), suggesting that both mutations arose as a result of ENU administration. Heterozygous N2 backcross progeny of the founder 31L and 100P heterozygous (DBA/2JJcl × C57BL/6JJcl) F1 males and wild-type C57BL/6JJcl females were backcrossed through the male and female lines to C57BL/6J for four generations (N3–N6) before heterozygotes with a predominantly C57BL/6J genetic background (average of 98.4375% at N6) were intercrossed to generate homozygous (31L/31L; 100P/100P), heterozygous (31L/+; 100P/+), and compound heterozygous (31L/100P) mutants for phenotypic testing, which were viable and grossly indistinguishable from their wild-type (+/+) littermates. 31L/+ (Rgsc1393) and 100P/+ (Rgsc1390) mice are available from the RIKEN BioResource Center (www.brc.riken.jp/lab/animal/en/gscmouse.shtml).
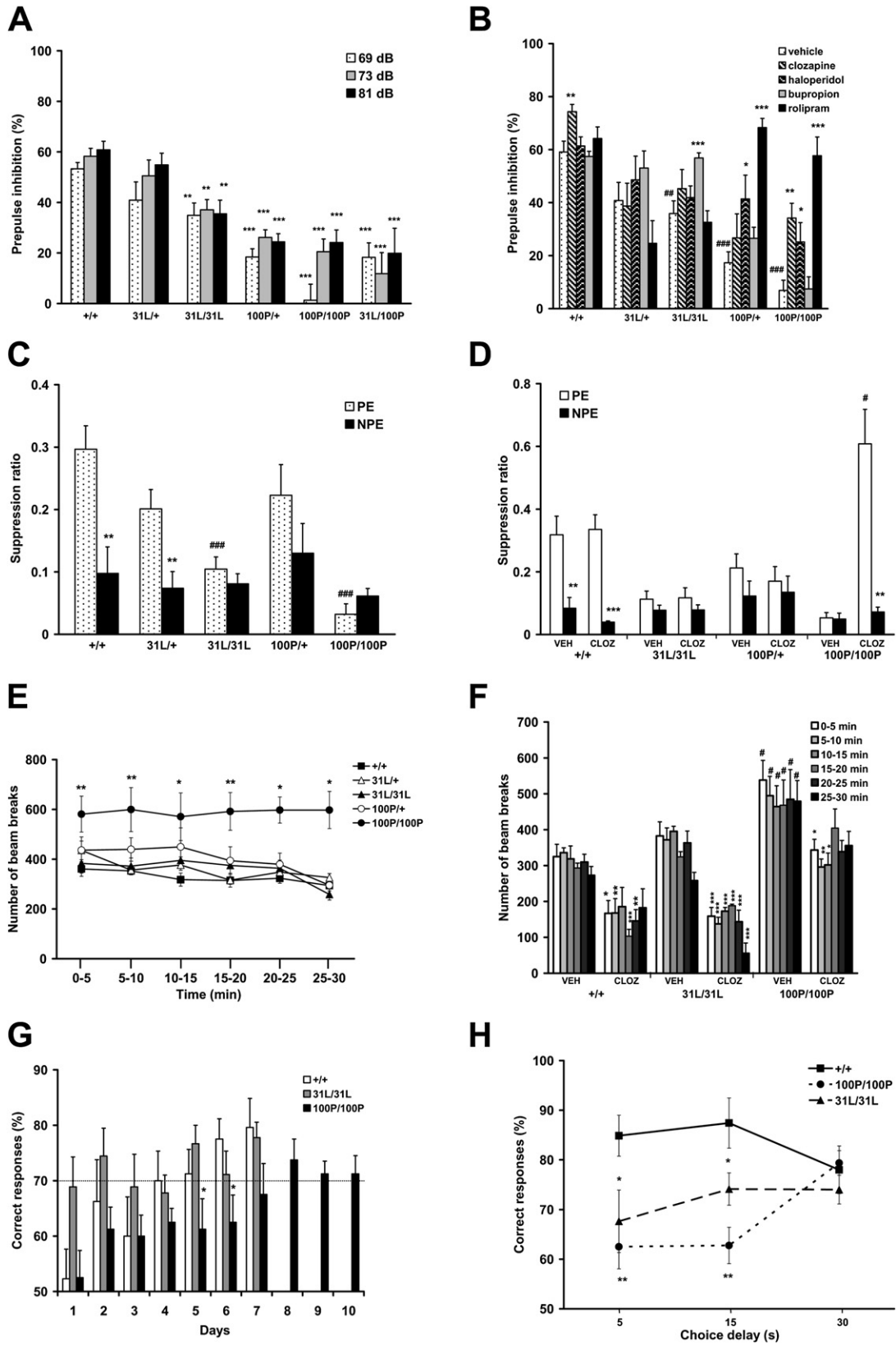
Schizophrenic-like Phenotype in 100P *Disc1* Mutant Mice

Deficits in attention and information processing are considered a central feature of schizophrenia, which lead to stimulus overload, cognitive fragmentation, and thought disorders (Perry and Braff, 1994). Prepulse inhibition (PPI) and latent inhibition are the most common methods to quantify information-processing deficits in schizophrenia with a reasonable amount of face, predictive, and construct validity (Geyer and Ellenbroek, 2003), and they can be used in both human and animal experiments (Arguello and Gogos, 2006).

PPI is the degree to which the acoustic startle response is reduced when the startle-eliciting stimulus is preceded by a brief low-intensity stimulus that does not elicit a startle response. We found that 100P/100P, 100P/+, 31L/31L, and 31L/100P mice had lower PPI than +/+ mice (Figure 2A). In the absence of a prepulse, the amplitude of the acoustic startle response was lower in 100P/100P, 100P/+, and 31L/100P mice than in +/+ mice (see Figure S1 in the Supplemental Data available with this article online), while 100P/100P mice also had lower startle reactivity to a wide range of startle intensities (Figure S1). We found no correlation between startle response and PPI in 100P/100P mice (r = 0.16, p = 0.14). The lower PPI of 100P/100P and 100P/+ mice was not a result of impaired hearing, as the auditory brainstem response (ABR) threshold at 16 kHz was not different between 100P/100P (40 ± 2.6) and +/+ mice (46 ± 5.1).

The typical antipsychotic haloperidol, a dopamine D2 receptor antagonist, and the atypical antipsychotic clozapine, an antagonist of both dopamine and serotonin receptors, both partially alleviated the pronounced deficit of 100P/100P mice. Clozapine also increased PPI in

Figure 1. Missense Mutations in Mouse *Disc1*

(A) Alignment of the predicted disrupted in schizophrenia 1 protein sequences of mouse Disc1 (ENSMUSP00000074147) and human DISC1 (ENSP00000295051) using the Clustal W 1.81 program. Residues identical between mouse and human have a gray background. The codon mutated in each *Disc1* mutant mouse line is shown in red text and indicated by a red arrow. Nonsynonymous SNPs in human *DISC1* associated with increased risk for major depression (Hashimoto et al., 2006), bipolar disorder (Maeda et al., 2006), schizophrenia (Callicott et al., 2005; Cannon et al., 2005), or schizoaffective disorder (Hodgkinson et al., 2004) are shown in green text and indicated by green arrows. The breakpoint of the translocation that segregates with psychiatric illness in a large Scottish family (Millar et al., 2000) is indicated by a vertical bar (|) labeled BP. Dashes indicate alignment gaps. Numbers to the right of the alignment show the amino acid position of the corresponding aligned sequence. Exons are indicated by alternating black and blue text.

(B) DNA sequence chromatograms showing point mutations in *Disc1* Exon 2. Transversion 127A→T is predicted to convert residue 31 from CAG glutamine (Gln) to CTG leucine (Leu). Transition 334T→C is predicted to convert residue 100 from CTC leucine (Leu) to CCC proline (Pro).

100P/+ and +/+ mice, but 31L/+ and 31L/31L mice did not respond to either antipsychotic (Figure 2B). The antidepressant bupropion, a dopamine and norepinephrine reuptake inhibitor, abolished the mild PPI deficit of 31L/31L mice but was not effective against the severe PPI impairment of 100P/100P or 100P/+ mice (Figure 2B). Conversely, the PDE4 inhibitor rolipram increased the PPI of 100P/100P and 100P/+ mice to the level of +/+ mice but did not reverse the milder PPI deficit of 31L/31L mice (Figure 2B). None of the drugs affected the acoustic startle response (Table S1).

Latent inhibition (LI) is the phenomenon by which prior exposure to a nonrewarding conditioned stimulus decreases the salience of the conditioned stimulus (CS) when it is later paired with an unconditioned stimulus (US) (Weiner, 2003). The drinking performance of mutant mice was normal, as genotypes neither differed in drinking behavior during 5 days of training prior to testing (Figure S1) nor in time to complete licks 50–75 before CS onset during testing. LI was disrupted in 100P/100P, 100P/+, and 31L/31L mice pre-exposed to the CS (Figure 2C). A strong CS-US association was exhibited

by non-pre-exposed mice of all genotypes (Figure 2C), suggesting that the LI disruption was not due to a gross cognitive deficit but a specific information-processing deficit. The administration of clozapine abolished the disruption of LI in 100P/100P mice but had no effect on LI in the other genotypes (Figure 2D).

In a novel, open field, 100P/100P mice displayed greater horizontal locomotion throughout the 30 min testing period (Figure 2E), whereas vertical locomotion (rearing) was greater only during the first 10 min (Figure S1). No differences in locomotion were displayed by the other mutants. Clozapine-treated mice of all genotypes displayed lower horizontal locomotion than vehicle-treated counterparts (Figure 2F). In the elevated plus maze, a test of anxiety (Rogers and Cole, 1994), the duration of time on the open arms was not different between genotypes (Figure S1), suggesting that open field locomotion was not affected by altered anxiety levels.

We assessed working memory using a discrete paired-trial variable-delay T-maze task (Aultman and Moghaddam, 2001). When this task was previously applied to *Disc1* deletion carriers, they performed normally during training but showed a deficiency across all choice delay intervals in the working memory test (Koike et al., 2006). We found that 100P/100P mice required more training than +/+ and 31L/31L mice to reach 70% correct responses on 3 consecutive days (Figure 2G). In the working memory test, 31L/31L and 100P/100P mutants had fewer correct responses at shorter (5 s and 10 s) but not longer (30 s) choice delay intervals (Figure 2H). In the Morris water maze, a test of spatial learning and memory, the performance of 31L and 100P mutants was not different from that of +/+ mice (Figure S1), suggesting that reference memory was not affected by the missense mutations.

Depressive-like Phenotype in 31L *Disc1* Mutant Mice

In the forced swim test (FST), a well-established paradigm to detect depressive-like behavior in rodents (David et al., 2003), the duration of floating immobile on the surface of the water was increased in 31L/31L mutants (Figure 3A). As antidepressant drugs have been shown to decrease the duration of immobility (David et al., 2003), this measure is used as an index of behavioral despair. We found that bupropion and rolipram both reduced immobility time in +/+ mice, but only bupropion significantly reduced immobility time in 31L/31L mice (Figure 3B). In a 2 day FST procedure, a new cohort of 31L/31L, 31L/+, and +/+ mice all had greater immobility on day 2 than on day 1 (Figure S2), suggesting that all genotypes were exhibiting learned helplessness. However, 31L/31L mutants displayed higher

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Figure 2. Schizophrenic-like Behaviors in 31L and 100P Mutant Mice

(A) Prepulse inhibition of acoustic startle response. PPI assay using a combination of startle (120 dB) and three prepulse levels (69 dB, 73 dB, and 81 dB) in 31L/31L (n = 24), 31L/+ (n = 24), 100P/100P (n = 21), 100P/+ (n = 20), +/+ (n = 21), and 31L/100P (n = 10) mice. PPI is expressed as the mean percent reduction (±SEM) in startle amplitude at all three prepulses. Higher y axis values represent greater percent PPI. There were significant effects of genotype ($F_{(5,120)}$ = 11.17, p = 0.0009), prepulse intensity ($F_{(2,240)}$ = 4.92, p = 0.008), and genotype-prepulse interaction ($F_{(10,240)}$ = 2.70, p = 0.004). *p < 0.05; **p < 0.01; ***p < 0.001 versus +/+ mice.

(B) Pharmacological responses in PPI assay. Mean effects of clozapine (3 mg/kg), haloperidol (0.4 mg/kg), bupropion (4 mg/kg), and rolipram (0.5 mg/kg) on PPI (±SEM) in 31L/31L (n = 10–16), 31L/+ (n = 10–24), 100P/100P (n = 11–17), P/+ (n = 10–22), and +/+ (n = 11–16) mice. There were significant effects of genotype ($F_{(4,290)}$ = 23.40, p = 10^{-6}), drug ($F_{(4,290)}$ = 6.22, p = 0.00006), and genotype-drug interaction ($F_{(16,290)}$ = 5.16, p = 6 × 10^{-5}). *p < 0.05; **p < 0.01; ***p < 0.001 versus vehicle-treated mice within each genotype. ##p < 0.01; ###p < 0.001 versus vehicle-treated +/+ mice.

(C) Latent inhibition of fear conditioning. Mean suppression ratios (±SEM) of 31L/31L (n = 9 PE, 8 NPE), 31L/+ (n = 16 PE, 16 NPE), 100P/100P (n = 7 PE, 7 NPE), 100P/+ (n = 11 PE, 11 NPE), and +/+ (n = 13 PE, 8 NPE) mice pre-exposed (PE) and non-pre-exposed (NPE) to the CS. LI is indicated by a lower suppression of drinking (higher suppression ratio) in PE compared to NPE mice, and was exhibited only by 31L/+ and +/+ mice. There were significant effects of genotype ($F_{(4,96)}$ = 4.22, p = 0.003) and pre-exposure ($F_{(1,96)}$ = 11.21, p = 0.001). There was no difference between genotypes in the time to complete licks 50–75 (period A; before CS onset). *p < 0.05; **p < 0.01 versus PE mice within each genotype. ### p < 0.001 versus PE +/+ mice.

(D) Clozapine response in LI assay. Mean suppression ratios (±SEM) of vehicle- and clozapine-treated (PE, NPE) mice: 31L/31L (n= 6, 6 and n = 6, 6), 100P/100P (n = 6, 6 and n = 7, 6), 100P/+ (n = 6, 6 and n = 11, 9), and +/+ (n = 7, 10 and n = 8, 7). Clozapine rescued the LI deficit of 100P/100P mice only. Clozapine (3 mg/kg, 30 min, i.p.) was administered in both the pre-exposure and conditioning stages. There were significant effects of genotype ($F_{(3,102)}$ = 3.1, p = 0.03), drug ($F_{(1,102)}$ = 7.8, p = 0.006), pre-exposure ($F_{(1,102)}$ = 39.5, p = 0.0001), and genotype × drug × pre-exposure interactions ($F_{(3,102)}$ = 7.7, p = 0.0001). **p < 0.01; ***p<0.001 versus PE mice within each genotype and drug treatment. #p < 0.05 versus vehicle-treated PE-100P/100P mice.

(E) Horizontal locomotion in the open field. Mean number of beam breaks (±SEM) in 5 min bins by 31L/31L (n = 6), 31L/+ (n = 17), 100P/+ (n = 7), 100P/100P (n = 9), and +/+ (n =14) mice. There were significant effects of genotype ($F_{(4,48)}$ = 4.0, p = 0.008) and time interval ($F_{(5, 240)}$ = 6.7, p = 0.00007). Horizontal locomotion was higher in 100P/100P mutants at all time points (p < 0.05 at 10–15, 20–25, and 25–30 min, p < 0.01 at 0–5, 5–10, and 15–20 min). 100P/+, 31L/+, and 31L/31L mutants were not significantly different from +/+ mice. *p < 0.05; **p < 0.01 versus +/+ mice.

(F) Clozapine effect on open field horizontal locomotion. Mean number of beam breaks (±SEM) in 5 min bins by vehicle- and clozapine-treated 31L/31L (n = 6, 6), 100P/100P (n = 8, 10), and +/+ (n = 7, 8) mice. There was a significant effect of genotype ($F_{(1, 36)}$ = 27.8, p = 6 × 10^{-6}), drug ($F_{(1, 36)}$ = 14.2, p = 2.8 × 10^{-5}), and genotype × time interval interaction ($F_{(10, 180)}$ = 2.3, p = 0.01). Clozapine reduced the horizontal locomotion in the open field of all genotypes. *p < 0.05; **p < 0.01; ***p < 0.001 versus vehicle-treated mice within each genotype. #p < 0.05 versus vehicle-treated +/+ mice.

(G) T-maze alternation training. Mean percentage of correct responses (±SEM) during the discrete paired-trial delayed alternation training by 31L/31L (n = 9), 100P/100P (n = 8), and +/+ (n = 8) mice. There was a significant effect of genotype ($F_{(2,22)}$ = 7.56, p = 0.003) on correct responses. Note that the y axis starts at 50% correct responses, which represents the baseline response accuracy expected by chance. *p < 0.05 versus +/+ mice.

(H) Working memory performance with different delay intervals. Mean percentage of correct responses (±SEM) by 31L/31L (n = 9), 100P/100P (n = 8), and +/+ (n = 8) mice in the working memory test with choice delay intervals of 5 s, 15 s, and 30 s. There was a significant effect of genotype ($F_{(2,22)}$ = 9.6, p = 0.001) on correct responses. *p < 0.05; **p < 0.01 versus +/+ mice.
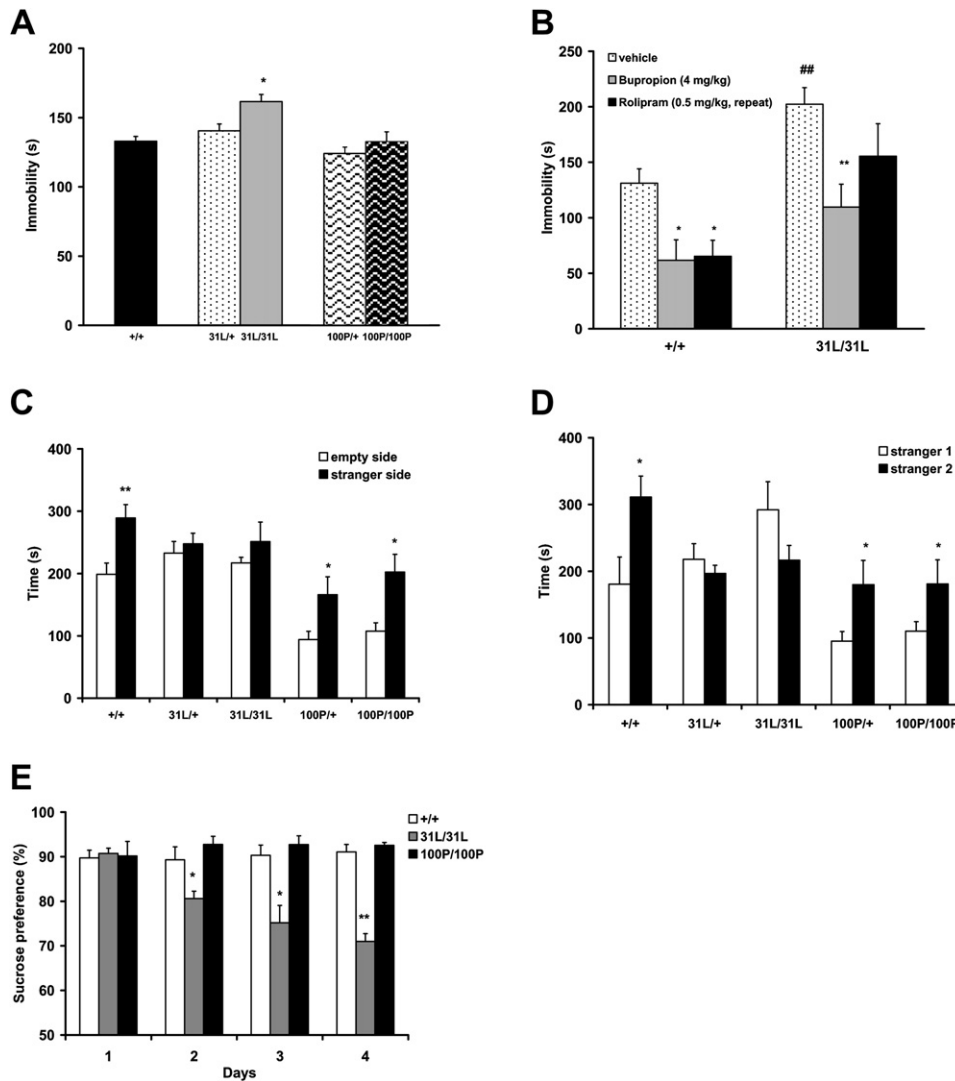
Figure 3. Behaviors of 31L Mutants Analogous to Symptoms of Depression

(A) Forced swim test. Mean duration of immobility (±SEM) of 31L/31L (n = 10), 31L/+ (n = 20), 100P/100P (n = 13), 100P/+ (n = 21), and +/+ (n = 27) mice. There was a significant effect of genotype ($F_{(4,86)}$ = 3.3, p = 0.02). Immobility time was longer in 31L/31L mice. *p < 0.05 versus +/+ mice.

(B) Pharmacological responses in the FST. Mean effects of bupropion (acute administration; 4 mg/kg, 30 min, i.p.) and rolipram (daily administration for 8 days; 0.5 mg/kg, 30 min, i.p.) on duration of immobility (±SEM) in 31L/31L (n = 7, 9) and +/+ (n = 6, 8) mice. There were significant effects of drug ($F_{(2,38)}$ = 5.1, p = 0.01) and genotype ($F_{(1,38)}$ = 10.6, p = 0.0003). Bupropion, but not rolipram, significantly decreased immobility duration in 31L/31L mice (p = 0.01). However, both drugs had antidepressant effects on +/+ mice. *p < 0.05, **p < 0.01 versus vehicle-treated mice within each genotype. ##p < 0.01 versus vehicle-treated +/+ mice.

(C) Sociability. Mean duration of time (±SEM) in the chamber with the stranger ("stranger side") than in the opposite chamber ("empty side"). Unlike +/+ (n = 6), 100P/+ (n = 7), and 100P/100P (n = 7) mice, 31L/+ (n = 8) and 31L/31L (n = 6) mice failed to demonstrate a preference for social proximity by spending significantly more time in the stranger side. There were significant effects of genotype ($F_{(4, 28)}$ = 9.62, p = 5.7 × 10^{-5}) and presence of "stranger 1" ($F_{(1, 28)}$ = 4.1 × 10^{-6}). *p < 0.05, **p < 0.01 versus empty side within each genotype.

(D) Social novelty. Mean duration of time (±SEM) in the chamber with the unfamiliar mouse from the sociability phase ("stranger 1") and in the opposite chamber with a new unfamiliar mouse ("stranger 2"). Unlike +/+ (n = 6), 100P/+ (n = 7), and 100P/100P (n = 7) mice, 31L/+ (n = 8) and 31L/31L (n = 6) mice failed to demonstrate a preference for social novelty by spending significantly more time in the chamber with stranger 2, rather than the more familiar stranger 1. There were significant effects of genotype ($F_{(4, 28)}$ = 7.88, p = 0.0002), presence of new partner (stranger 2) ($F_{(1, 28)}$ = 5.97, p = 0.02), and genotype × presence of new partner interaction ($F_{(4, 28)}$ = 4.79, p = 0.005). *p < 0.05 versus "stranger 1" side within each genotype.

(E) Sucrose consumption test. Preference for a 10% sucrose solution over water is shown as the mean percentage (±SEM) of sucrose solution ingested relative to the total amount of liquid consumed. There were significant effects of genotype ($F_{(2,17)}$ = 28.43, p = 0.0001), test day ($F_{(3,51)}$ = 5.39, p = 0.003), and genotype × test day interaction ($F_{(6, 51)}$ = 8.35, p = 5 × 10^{-6}) on sucrose preference. 31L/31L (n = 6) mice consumed significantly less of the sucrose solution relative to water than +/+ (n = 8) and 100P/100P (n = 6) mice on day 2 (p < 0.01), day 3 (p < 0.05), and day 4 (p < 0.01), consistent with a lower responsiveness to rewards. *p < 0.05, **p < 0.01 versus +/+ mice.

immobility than +/+ and 31L/+ mice on both days (Figure S2), suggesting an effect of despair rather than fatigue.

Next, we investigated whether the depressive-like behavior of 31L/31L mice in the FST was also exhibited in the less-aversive social interaction (Moy et al., 2004) and reward responsiveness (Pothion et al., 2004) tests, deficits in which are analogous to key symptoms of depression (American Psychiatric Association, 1994). In the sociability phase of the social interaction test, we found that +/+ mice and 100P mutants spent more time in a chamber containing an unfamiliar mouse inside a cylinder than in the opposite chamber containing an empty cylinder, whereas 31L mutants did not (Figure 3C). In the social novelty phase of the test, +/+ mice and 100P mutants demonstrated a shift in preference, spending more time in a chamber containing a new unfamiliar mouse ("stranger 2") than in the chamber containing the original unfamiliar mouse from the sociability phase ("stranger 1"), whereas again 31L mutants did not (Figure 3D). The lower social interaction of 31L mutants was not a result of diminished olfaction, as we found that the time required to find buried food in an olfaction test was not different between genotypes (Figure S2).

We tested reward responsiveness by measuring sucrose consumption. Low responsiveness to rewards, such as a sweet taste, is comparable to anhedonia, the core symptom of the melancholic subtype of depression (Willner, 1997). When presented with two drinking bottles, one containing water and the other containing 10% sucrose, 31L/31L mice consumed significantly less of the sucrose solution relative to water than +/+ and 100P/100P mice on the last 3 days of the 4 day test (Figure 3E), which is consistent with a lower responsiveness to rewards. We found no evidence for a difference in taste sensitivity in 31L/31L mice (Figure S2).

Reduced Brain Volume in 31L and 100P *Disc1* Mutant Mice

Structural and functional imaging studies have established that brain anatomical abnormalities are a consistent feature of schizophrenia (Ross et al., 2006). Recent evidence suggests that this is also true in depression (Meyer-Lindenberg and Weinberger, 2006) and bipolar affective disorder (Hasler et al., 2006). We therefore examined 31L and 100P mutants for evidence of structural differences in the brain. Magnetic resonance imaging (MRI) revealed reductions in overall brain volume of 13% in 100P/100P and 100P/+ mice and 6% in 31L/31L and 31L/+ mice (Figure 4A). These overall reductions were accompanied by tissue contraction in the cortex, entorhinal cortex, thalamus, and cerebellum of 100P and 31L mutants (Figures 4B and 4C).

DISC1 Protein Levels in 31L and 100P *Disc1* Mutant Brain

To assess the effects of the 31L and 100P mutations on DISC1 protein levels in the brain, we measured DISC1 im-

munoreactivity in brain extracts from 31L/31L, 100P/100P, and +/+ mice. An antibody that recognizes an epitope at the C-terminal region of DISC1, unaffected by both missense mutations, detected major bands of ~71, 75, and 105 kDa, plus several minor bands, in all genotypes (Figure S3). The three major bands match comparably sized bands previously detected in adult mouse brain using different DISC1 C-terminal antibodies, the ~105 kDa band likely representing full-length DISC1 (Schurov et al., 2004; Ishizuka et al., 2006). This suggests that both *Disc1* mutant lines express all of the major DISC1 isoforms. Densitometry analysis of the ~71, 75, and 105 kDa signals revealed no significant differences between genotypes.

Subcellular Distribution of DISC1 and PDE4B in *Disc1* Mutant Brain

To examine effects of the 31L and 100P mutations on the subcellular distribution of DISC1, we prepared subcellular fractions from +/+, 31L/31L, and 100P/100P mouse brains. Immunoblotting of synaptosomes and postsynaptic density (PSD) fractions revealed that the ~71 and 75 kDa DISC1 isoforms were predominantly expressed in purified synaptosomes, PSD1, and PSD2 fractions, but not enriched within the core PSD fraction, PSD3 (Figure S3). In addition, an immunoreactive band at ~200 kDa was detected in all PSD fractions (Figure S3). This ~200 kDa species may represent an SDS-resistant DISC1 complex or an additional DISC1 isoform. We also investigated the distribution of PDE4B in these fractions, since we have previously demonstrated a robust interaction between PDE4B and DISC1 in human cells (Millar et al., 2005). PDE4B was associated with synaptosomes and all PSD fractions (Figure S3). In addition, we analyzed P3 (microsomes and light membranes) and ER-G (a mixture of ER, Golgi, and plasma membranes) fractions, and immunoblotting analysis detected the ~71 and 75 kDa DISC1 isoforms in both fractions (Figure S3). PDE4B was also abundant in both fractions (Figure S3). We did not attempt to analyze nuclear fractions due to the unavoidable contamination of this fraction with cellular debris. There was no detectable difference in distribution of DISC1 or PDE4B in any of the fractions tested from +/+, 31L/31L, and 100P/100P mice.

Reduced PDE4B Binding to Mutant DISC1

The N-terminal head domain of DISC1 is important for interaction with PDE4B, and we previously demonstrated that amino acids 220–283 are involved in binding (Millar et al., 2005). However, a peptide array profiling approach has since demonstrated that multiple regions of human DISC1 act as contact sites for PDE4B, including additional sites within the head domain that encompass the positions of the mouse 31L and 100P mutations (M.D.H., unpublished data). We therefore investigated the possibility that these missense mutations influence binding between DISC1 and PDE4B. When DISC1 and PDE4B were exogenously coexpressed in HEK293 cells, we found that both mutations significantly reduced binding between DISC1
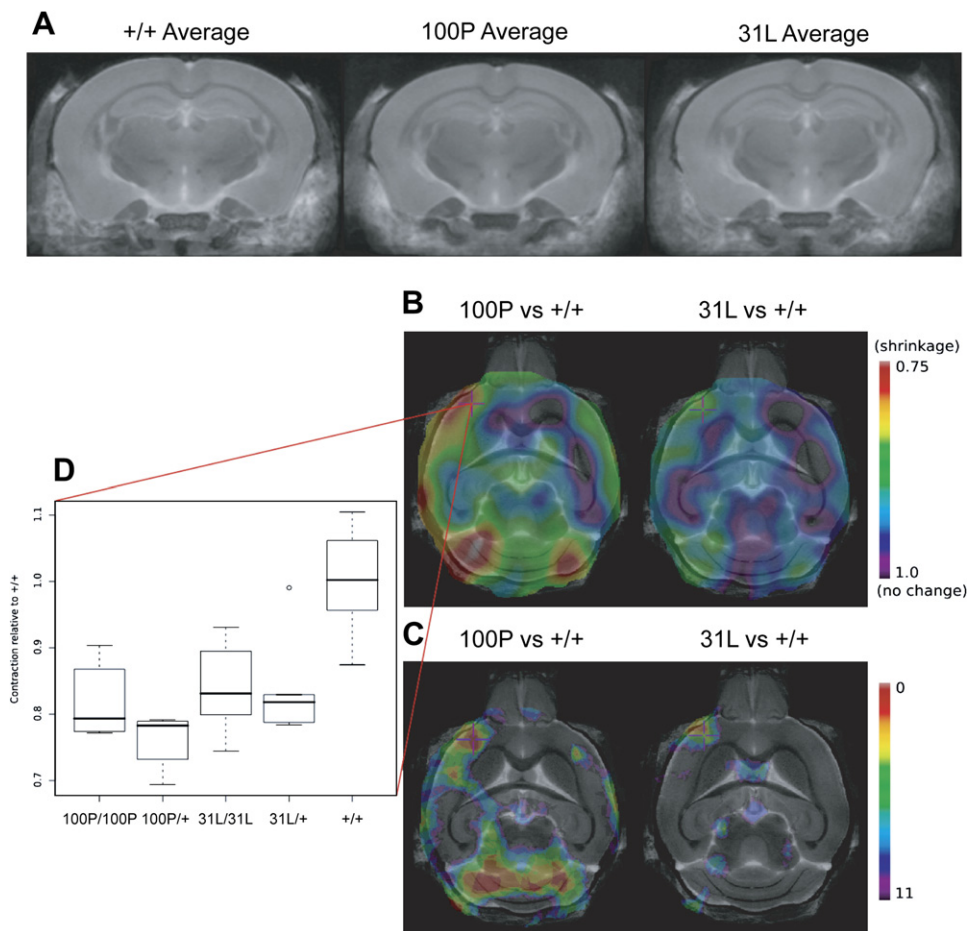
Figure 4. Reduced Brain Volume in 31L and 100P Mutant Mice

(A) Composite mean brains of the wild-type (+/+, n = 6), 100P (100P/100P, n = 6; 100P/+, n = 6), and 31L (31L/31L, n = 6; 31L/+, n = 6) groups after nonlinear registration. The overall volume of the 100P and 31L mutant brains was lower by 13% (U = 7, p = 0.019) and 6% (U = 10, p = 0.05), respectively.

(B) Relative contraction of brain regions. Differences in Jacobian determinants in the 31L (p = 0.046) and 100P (p = 0.006) groups as compared to the wild-type group. The contraction around the cortex is noticeable while the white matter tracts remain unchanged. The maps feature a spatially similar pattern between the two groups, though the 100P group has greater contraction.

(C) Thresholded results from the Mann-Whitney U test. Values of U (0–11) are represented by colors indicated in the spectrum to the right.
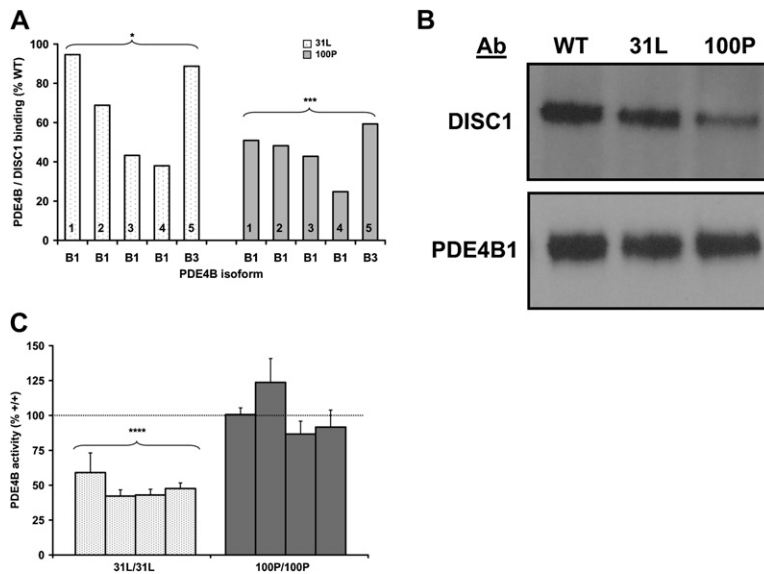
(D) Box plot of tissue contraction (normalized to the mean Jacobian of the +/+ group) of a single voxel in the cortex. The pattern of tissue contraction is clearly evident in the graph, as is the relative similarity between homozygotes and heterozygotes for each mutation.

and PDE4B, with the 100P mutation exerting the greatest effect (Figure 5). We observed the same outcome with the long isoforms PDE4B1 and PDE4B3. However, the degree of binding was variable, particularly with the 31L mutation, suggesting that mutant DISC1 binding to PDE4B is influenced by the effects of unknown fluctuating cellular factors.

Lower PDE4B Activity in 31L *Disc1* Mutant Brain

We previously reported that the head domain of DISC1 binds to the UCR2 domain of PDE4B in a cAMP-dependent fashion (Millar et al., 2005) and proposed a model in which DISC1 binds to an unphosphorylated low-activity form of PDE4B that is released as a PKA-phosphorylated

high-activity form in response to cAMP upregulation, thus providing a negative-feedback mechanism to modulate cAMP levels (Millar et al., 2005). Given this dynamic interaction and the different responses of 31L and 100P mutants to the PDE4 inhibitor rolipram, we determined the relative amounts of DISC1 and PDE4B protein and PDE4B catalytic activity in 31L/31L, 100P/100P, and +/+ brain extracts. Intriguingly, although we saw no difference in the amount of PDE4B protein between *Disc1* mutant and wild-type brains (Figure S3), we did see a striking 50% reduction in PDE4B activity specifically in 31L/31L brains (Figure 5), indicating that the activity status of PDE4B is altered in this mutant line. This reduction in PDE4B activity is consistent with the resistance of 31L

Figure 5. Analysis of PDE4B

(A and B) Reduced PDE4B binding to mutant DISC1.

(A) Association between exogenously expressed PDE4B isoforms and mutant DISC1 expressed as percentage of wild-type. Five independent binding assays were performed with each mutant in parallel, using PDE4B1 (B1) or PDE4B3 (B3). Numbers within bars refer to each independent experiment. The degree of binding was variable between experiments for each mutant, but both mutants showed similar alterations in binding within each experiment. Binding of PDE4B isoforms to 31L and 100P mutant DISC1 was significantly reduced. *p<0.05, ***p<0.001.

(B) Typical blot of PDE4B1 immunoprecipitations probed with anti-DISC1 (upper) and pan-PDE4B (lower) antibodies. The bands shown correspond to the predicted 100 kDa DISC1 and 80 kDa PDE4B1 forms.

(C) Lower PDE4B activity in 31L mutant brain. Activity of PDE4B in the brains of 31L/31L (n = 4) and 100P/100P (n = 4) mice. Activity measurements are means (±SEM) for three separate experiments. Results are expressed as a percentage of wild-type control (n = 4) to minimize interexperiment variation. Mean PDE4B activity in 31L/31L brain tissue was reduced by 47.98% ± 6.70%. ****p < 0.00001.

mutants and the contrasting sensitivity of 100P mutants and wild-type mice to treatment with rolipram (Figure 2B).

DISCUSSION

ENU Induced Mutations in *Disc1*, Predicted Functional Consequences, and Pathophysiological Mechanisms

In our screen of *Disc1* Exon 2, two mutations were found in 1686 mice, a rate comparable with the reported genome-wide average for a functional mutation in any single locus of 1 in 500–1500 F1 mice (Justice et al., 1999). Based on this average rate, each founder *Disc1* mutant was expected to be heterozygous for ∼24 additional functional mutations randomly distributed across the ∼25,000 genes in the mouse genome. By backcrossing each *Disc1* mutation to the C57BL/6J strain for five generations (N2–N6), we reduced the expected number of additional heterozygous mutations in each mutant line to 0.75 (Michaud et al., 2005). Given that only 25% of these 0.75 additional mutations are expected to be carried by both mice of a sixth generation intercross breeding pair (Michaud et al., 2005), it is highly unlikely that any of their progeny used for phenotypic testing would be homozygous for a mutation in any gene other than *Disc1*. Moreover, the use of multiple breeding pairs having different combinations of additional mutations and the free segregation of such unlikely mutations across all *Disc1* genotypes within their progeny effectively eliminates the chance of falsely associating the observed mutant phenotypes with the missense mutations in *Disc1*.

These mutations, on a C57BL/6J background, are thus quite distinct from the spontaneous 25 bp deletion in Exon 6 of *Disc1* discovered in the 129S6/SvEv substrain (Koike et al., 2006), and subsequently in all other 129 mouse substrains (Clapcote and Roder, 2006). Koike et al. (2006) reported impairment in a single working memory test, but no gross brain morphology, altered open field activity, or PPI deficit when the *Disc1* deletion was crossed onto a C57BL/6J background. This preliminary molecular and behavioral characterization merits further analysis, particularly as a recent collaborative study using multiple validated DISC1 antibodies suggests that all but one possible isoforms of DISC1 are intact and normally expressed from the deletion allele (Ishizuka et al., 2007).

The three-dimensional structure of DISC1 has yet to be solved, but the nature of the Q31L and L100P amino acid exchanges gives clues to their likely effects. Glutamine (Q) is hydrophilic and would normally be found on the outer surface of a protein. This is probably true in the case of Q31, as it is immediately adjacent to a predicted bipartite nuclear localization signal (RRRLTRRP). The 127A→T transversion resulted in Q31 being replaced by a leucine (L), a hydrophobic amino acid that would normally be expected to be located within the interior of a protein. The 334T→C transition resulted in L100 being replaced by a proline (P), a hydrophobic amino acid whose ring structure is known to cause a sharp transition in polypeptide chain direction, which can either interrupt the formation of or terminate any regular structural feature. Thus, such missense mutations most likely result in distinct structural alterations in DISC1.

These missense *Disc1* mutants may therefore model the putative (but yet to be formally established) missense mutations, such as Ser704Cys, that are thought to account for a proportion of the liability to schizophrenia and related

psychiatric disorders attributable to DISC1 variation (Porteous et al., 2006). To the best of our knowledge, however, there are no directly comparable missense mutations in Exon 2 of human *DISC1*.

Neuroanatomical Abnormalities

Neuroanatomical abnormalities have been consistently reported following brain imaging of schizophrenics, bipolar subjects, and their "high-risk" relatives (Strakowski et al., 1999; McIntosh et al., 2007; Ross et al., 2006), and there is preliminary evidence awaiting validation that specific *DISC1* polymorphisms associate with structural and functional brain alterations in schizophrenic and normal subjects (Callicott et al., 2005; Cannon et al., 2005; Hashimoto et al., 2006). Consistent with this, MRI detected neuroanatomical abnormalities in both *Disc1* mutant lines. The reductions in overall brain volume of 6% in 31L mutants and 13% in 100P mutants (Figure 4) correspond well with observations of small but significant reductions in brain volume in patients with schizophrenia (Lawrie and Abukmeil, 1998; Wright et al., 2000). The similarity in the magnitude of the brain volume and PPI reductions associated with each mutation suggests that these effects are interrelated. 31L and 100P mutants both showed contraction of the cortex, entorhinal cortex, thalamus, and, particularly, the cerebellum (Figure 4), all of which are brain regions previously implicated in the pathophysiology of schizophrenia (Harrison and Weinberger, 2005; Konarski et al., 2005; Ross et al., 2006). The contraction of the cerebellum in both *Disc1* mutants provides support for the role of this brain region in the pathophysiology of psychiatric disease, as suggested by preliminary evidence that patients with depression and schizophrenia may share similar structural (volumetric reductions) and functional (baseline hyperactivity) abnormalities in the cerebellum (Konarski et al., 2005). Our observations are also consistent with the evidence that *Disc1* is developmentally regulated and expressed in all of the affected brain regions (Schurov et al., 2004; Ross et al., 2006).

Behavioral Abnormalities

Deficits in sensory processing of distracting incoming information and excessive stimuli may contribute to the cognitive impairments and auditory hallucinations that characterize many schizophrenic patients (Swerdlow et al., 1994). LI is believed to measure decremental attention, which is the ability to ignore irrelevant stimuli. Schizophrenics exhibit characteristic abnormalities in LI, especially when they are acutely symptomatic (Rascle et al., 2001; Williams et al., 1998). PPI is a common measure of sensorimotor gating that can be applied to both humans and mice (Gottesman and Gould, 2003; Arguello and Gogos, 2006). PPI is characteristically decreased in patients with schizophrenia (Braff et al., 1992; Grillon et al., 1992), in schizotypal personality disorder (Cadenhead et al., 1993), and also in psychosis-prone individuals (Braff et al., 2001). We observed significant and different deficits in these and other behavioral measures in the 31L and

100P mutant mice, as summarized in Table 1. The effects on sensory processing, as measured by PPI and LI, were pronounced in 100P/100P and 100P/+ mice and modest, but still significant, in 31L/31L mutants (Figure 2). The pronounced PPI deficit of compound heterozygous 100P/31L mice suggests that the 100P mutation is dominant over both the 31L and wild-type alleles. The capacity of antipsychotics and rolipram to improve PPI in 100P/100P mutants without effects on startle response (Table S1) and the lack of a correlation between startle response and PPI in these mice suggest that their low PPI is not merely a function of low startle. Indeed, several inbred mouse strains with a low startle response are reported to have high PPI levels (Paylor and Crawley, 1997). The impairment of both *Disc1* missense mutants in a working memory test (Figure 2H) that was the only behavioral paradigm to reveal a deficiency in *Disc1* deletion carriers (Koike et al., 2006) suggests that working memory might be particularly sensitive to *Disc1* mutation. The hyperactivity of 100P/100P mice in the open field resembled the open field behavior of wild-type rodents given amphetamine (Ralph et al., 2001).

In the FST, only 31L homozygotes exhibited reduced mobility (Figure 3). Upon further examination, 31L homozygotes also exhibited reduced sociability and social novelty and reduced reward responsiveness (Figure 3), behavioral paradigms that model anhedonia (Cryan and Holmes, 2005), a key depression symptom characterized by markedly diminished interest or pleasure in everyday activities (American Psychiatric Association, 1994).

Pharmacological Responses

The PPI and LI deficits in 100P homozygotes responded to both typical (haloperidol) and atypical (clozapine) antipsychotics, consistent with a schizophrenic-like phenotype (Table 1). The PDE4 selective inhibitor rolipram also substantially rescued the PPI deficit in 100P/100P and in 100P/+ mice but did not alter PPI in 31L mutants or wild-type mice. Conversely, the biogenic amine reuptake inhibitor bupropion was the only drug evaluated that increased PPI in 31L homozygotes but did not alter PPI in 100P mutants or wild-type mice. Bupropion and rolipram both reduced the floating behavior of wild-type mice in the FST, but intriguingly only bupropion significantly reduced the floating behavior of 31L homozygotes. The response to antipsychotics and resistance to bupropion in 100P mutants and the converse in 31L mutants provides further support for the notion that the former display schizophrenic-like and the latter depressive-like phenotypes.

Rolipram is a potent, highly selective inhibitor of PDE4, the enzyme that specifically catalyzes the hydrolysis of cAMP (Houslay et al., 2005), a second messenger in most neuromodulatory systems in the brain. Thus, the administration of rolipram increases cAMP levels in brain tissue, which is thought to have behavioral effects via the consequent upregulation of cAMP signaling in the hippocampus and cerebral cortex (Scheider, 1984; Wachtel, 1983). However, the 50% reduction in cAMP hydrolyzing

Table 1. Summary of Phenotypes of 31L and 100P *Disc1* Mutant Mice

Phenotype	31L 31L/31L	31L/+	100P 100P/100P	100P/+
Anxiety (elevated plus-maze)	=	=	=	=
Horizontal activity	=	=	>>	=
Vertical activity	=	=	>	=
Prepulse inhibition (PPI)	<	=	<<	<<
Acoustic startle response	=	=	<	<
Startle reactivity	=	=	<	=
Auditory brainstem response	n/a	n/a	=	n/a
Latent inhibition (LI)	<<	=	<<	<
Working memory (T maze)	<	n/a	<<	n/a
Spatial learning and memory (Morris water maze)	=	=	=	=
Forced swim immobility (FST)	>	=	=	=
Sociability and social novelty	<	<	=	=
Sucrose consumption	<	n/a	=	n/a
Olfactory function	=	=	=	=
Taste sensitivity	=	n/a	n/a	n/a
Brain volume	<	<	<<	<<
PDE4B activity	<<	n/a	=	n/a

Drug Treatment		31L/31L	31L/+	100P/100P	100P/+
PPI	Clozapine	=	=	+	=
	Haloperidol	=	=	+	=
	Rolipram	=	=	+++	++
	Bupropion	+++	=	=	=
LI	Clozapine	=	=	+	=
Horizontal activity	Clozapine	+++	n/a	++	n/a
FST	Bupropion	++	n/a	n/a	n/a
	Rolipram	=	n/a	n/a	n/a

= no significant difference; < lower than +/+; << much lower than +/+; > greater than +/+; >> much greater than +/+; +, ++, +++ positive effect ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively); n/a not applied.

activity of brain PDE4B, coupled with the rolipram-insensitivity of 31L mutants, suggests that increased cAMP levels are likely a contributory factor to the depressive-like behavior of 31L/31L mice. Moreover, the ability of rolipram to reverse the PPI deficits in 100P mutants, which have normal levels of PDE4B enzymatic activity, suggests that modulation of PDE4B by DISC1 also underlies some behavioral abnormalities associated with schizophrenia. That various *dunce* mutants of the *Drosophila* PDE4 gene, each with a profound learning deficit, differ significantly in absolute levels of PDE activity and cAMP led to the conclusion that it is the ability to modulate cAMP, rather than the baseline amount of cAMP, which is key for normal memory formation in the fruit fly (Davis and Kiger, 1981). Our results suggest that the same is true in mice and by extension abnormal cAMP levels, increased

or decreased, may contribute to psychiatric illness in humans.

To our knowledge, there are no reports on the efficacy in mentally ill carriers of the original t(1;11) translocation (Blackwood et al., 2001) or other putative *DISC1* risk alleles of any medication, including the four drugs employed in our mouse study. However, the different pharmacological responses of the 31L and 100P mutant mice might provide clues to effective medications for these patient groups. Indeed, these mice could represent a model system to explore novel treatment and preventative strategies for certain symptoms of major mental illness.

Abnormal DISC1/PDE4B Interaction
DISC1 abundance and subcellular distribution are indistinguishable from normal in mutant brains, indicating

that altered DISC1 function, rather than expression, gives rise to the depressive-like and schizophrenic-like phenotypes of the mutant mice. Indeed, we have demonstrated that the 31L and 100P mutations each reduce PDE4B binding to DISC1, while the 31L mutation additionally results in reduced PDE4B activity, which is not attributable to altered PDE4B expression, suggesting that altered DISC1/PDE4B function is fundamentally related to the phenotypes of both mutants. However, these observations are at odds with our previous data implying that decreased binding of PDE4B to DISC1 is likely to result in increased phosphodiesterase activity (Millar et al., 2005). This discrepancy is perhaps partially due to our incomplete understanding of the biochemistry of DISC1/PDE4B binding. Indeed, we now appreciate that the interaction between these two proteins is far more complex than we previously anticipated, since multiple points of contact between human DISC1 and PDE4B have recently been identified (M.D.H., unpublished data). Such multipoint attachment allows DISC1/PDE4B complexes to show isoform-specific responses to elevated cAMP levels (S.M., D.J.P., J.K. Millar, and M.D.H., unpublished data). This emerging complexity indicates that the differential impact of the DISC1 mutations upon PDE4B binding, and possibly also activity, is likely to be crucially related to their position within distinct contact sites for PDE4B. Alternatively, the reduced PDE4B activity of 31L mutants may not be related to the reduced DISC1 binding but may instead involve the extracellular-regulated kinase (ERK) pathway that is known to directly regulate PDE4B catalytic activity by phosphorylation of its catalytic unit (Baillie et al., 2000). Intriguingly, it was recently demonstrated that ERK activation is modulated by DISC1 expression levels in cultured neurons (Hashimoto et al., 2006; Shinoda et al., 2007); thus, mutant DISC1 may influence ERK activity leading indirectly to reduced PDE4B activity in the 31L mutant. Although we can only speculate upon the molecular causes of the altered PDE4B binding and activity in the mutants, we have shown that both DISC1 and PDE4B are abundant in synaptosomes and postsynaptic density fractions, and consequently it is possible that the distinctive phenotypes of the 31L and 100P mutant mice may arise in part from altered synaptic cAMP signaling.

Conclusion

We have shown that two independent missense mutations in mouse *Disc1* elicit distinct physiological, pharmacological, neuroanatomical, and behavioral phenotypes, which when taken together are strikingly consistent with the emerging picture from clinical and basic studies of DISC1 as a common genetic and biologically plausible risk factor for major mental illness. These mouse models support both the neurodevelopmental role for DISC1 (Kamiya et al., 2005) and the proposed cAMP signaling role through modulation of PDE4 activity (Millar et al., 2005; Porteous et al., 2006). Moreover, by virtue of the distinct phenotypes of the 31L and 100P mutants, our

findings lend further credence to the growing recognition that schizophrenia and bipolar disorder share, at least in part, common genetic etiologies and thus underlying molecular mechanisms (Craddock and Owen, 2005). Our results in the mouse thus emphasize the importance of replicating, validating, and resolving inconsistencies within the current picture of various *DISC1* alleles and haplotypes associating with distinct clinical phenotypes and, indeed, normal variation in cognitive function and neurodevelopment (Hennah et al., 2006; Porteous et al., 2006). By virtue of the complexity of DISC1 interaction with proteins intimately involved in either neurodevelopment or neuromodulatory signaling (Camargo et al., 2007), we can predict that secondary genetic factors, epigenetic factors, or environmental factors will modify the phenotypic consequences of any given DISC1 variant. This is well illustrated by the original t(1;11) family, which included multiple cases of schizophrenia and multiple cases of affective disorder, with a minority of translocation carriers having no recorded signs or symptoms of major mental illness. However, all those translocation carriers tested, whatever their clinical status, showed a deficit in the P300 event-related potential, a brain electrophysiological measure of distractedness, characteristic of schizophrenia (Blackwood et al., 2001). The linked hypotheses that different DISC1 variants will predispose to different phenotypes and that the phenotype of any given DISC1 variant will be modulated by components within, upstream, and downstream of the DISC1 scaffold complex are now amenable to experimental testing. By extension of the ENU mutagenesis screen within Exon 2 and throughout the *Disc1* locus, there is scope too for extending the allelic series to further refine genotype-phenotype correlations that can inform upon these most complex, debilitating, and poorly treated disorders.

EXPERIMENTAL PROCEDURES

Mutation Screening
TCGE heteroduplex detection was used to screen 1686 ENU-induced mutant mice for mutations in Exon 2 of *Disc1*, as described (Sakuraba et al., 2005).

Mice
Animal procedures were approved by the Animal Management Committee of Mount Sinai Hospital and were conducted in accordance with the requirements of the Ontario Animals for Research Act 1971 and the Canadian Council on Animal Care. Behavioral tests were conducted on sex-balanced groups of experimentally naive mice at 12–16 weeks of age, except where stated. Neither sex effects nor litter effects were detected by ANOVA in any of the behavioral tests, so data from different sexes and litters were pooled and analyzed together. Mice were genotyped for the 31L mutation using a nuclease mismatch detection protocol (Till et al., 2004) or for the 100P mutation by the absence of an Hpy188III (New England BioLabs) restriction site.

Behavioral Testing
PPI was measured using acoustic startle, as described (Lipina et al., 2005). ABR thresholds were recorded using a previously reported algorithm (Zheng et al., 1999). LI was measured by comparing suppression of drinking in response to a noise by mice that previously received

zero (non-pre-exposed, NPE) or 40 noise exposures (pre-exposed, PE) followed by two noise/foot shock pairings, as described (Lipina et al., 2005). Horizontal and vertical activity was measured for 30 min in 5 min bins inside a clear Perspex arena (41 × 41 × 33 cm) directly illuminated and equipped with infrared beams to automatically record horizontal and vertical movements (model 7420/7430, Ugo Basile). The elevated plus maze test was conducted as described (Avgustinovich et al., 2000). The discrete paired-trial T-maze task was conducted as described (Aultman and Moghaddam, 2001) to measure working memory in male mice that had been food restricted and adapted to the T maze (two trials for 2 days for 5 min). Spatial learning and memory were assessed in the Morris water maze, using a modification of a previously described protocol (Clapcote et al., 2005; Supplemental Experimental Procedures). The FST was conducted as described (Zhang et al., 2002). The sociability test was conducted as described (Moy et al., 2004) using apparatus consisting of a black Perspex rectangular box (51 cm long × 25.5 cm wide × 23 cm tall) divided into three interconnected chambers, as described (Sankoorikal et al., 2006). Olfactory ability was assessed by measuring latency to find a buried piece of food, as described (Stowers et al., 2002). The consumption of water and sucrose solution was measured by weighing the drinking bottles, as described (Pothion et al., 2004). Selection of the 10% concentration was based the reported preference of C57BL/6J mice for a sucrose solution at this concentration (Pothion et al., 2004). In a control experiment, baseline water intake of individually housed mice was recorded every 48 hr over 10 days. Water bottles were then replaced every 2 days with one of six different concentrations of freshly made sucrose solution (0.5%, 1%, 2%, 4%, 8% and 16%, weight/volume concentration) or water (0%), as described (Sakic et al., 1996).

Drug Administration
Drugs were administered by i.p. injection in a volume of 10 ml/kg 30 min before the commencement of the behavioral procedure. Clozapine (3 mg/kg; Tocris) and haloperidol (0.4 mg/kg; Tocris) were each dissolved in saline (0.9% NaCl) containing 0.3% Tween 20 (Bio-Rad). Bupropion (4 mg/kg; Sigma) was dissolved in distilled water. Rolipram (0.5 mg/kg; Sigma) was dissolved in saline containing 10% DMSO. The clozapine dose was selected from our previous study of C57BL/6J mice (Lipina et al., 2005). The doses of bupropion, haloperidol, and rolipram were determined by reference to previous rodent studies (David et al., 2003; Egashira et al., 2005; Pouzet et al., 2005; Zhang et al., 2002). In the LI experiment, clozapine was administered in both the pre-exposure and conditioning stages. In the FST, we injected mice with rolipram daily for 8 days and commenced testing 30 min after the last injection.

Magnetic Resonance Imaging
A four-channel 7.0-Tesla MR scanner (Varian) with a 6 cm inner bore diameter gradient set was used to acquire anatomical images of brains within skulls. The 3D MR images were initially aligned to one another by automated linear registration, as described (Chen et al., 2006; Kovacevic et al., 2005). Owing to the limited number of mice in each genotype group, we combined homozygotes and heterozygotes from each mutant line into single 31L or 100P groups (n = 12) to increase statistical power in comparisons with the wild-type group (n = 6).

Immunoblotting and Subcellular Fractionation
Whole brain extracts or subcellular fractions were subjected to SDS-PAGE and then immunoblotting analyses by incubation with indicated antibodies overnight at 4°C. Dilution of primary antibodies in 1% skimmed milk was as follows: DISC1 C-terminal polyclonal antibody ZMD.488 (Invitrogen; 1:250 for whole brain; 1:2000 for fractions); mouse anti-PSD-95 monoclonal antibody, clone 7E3-1B8 (ABR; 1:5000); mouse anti-synaptophysin monoclonal antibody, clone SVP38 (Sigma; 1:10,000), sheep anti-pan PDE4B polyclonal antibody (1:30,000). Subcellular fractionations were carried out as described

(Ueda et al., 1979; Niethammer et al., 2000; Okabe et al., 2003), with a slight modification (Supplemental Experimental Procedures).

Binding Assays
Mutations Q31L and L100P were introduced into pRK5-HADisc1 (a gift from A. Sawa) using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene). Lysates were prepared from transfected HEK293 cells expressing various combinations of wild-type mouse DISC1 or DISC1 mutants (Q31L and L100P) and FLAG-tagged PDE4B1 or PDE4B3 (Millar et al., 2005). For immunoprecipitation experiments, mouse monoclonal anti-FLAG M2 (Sigma) or sheep polyclonal pan-PDE4B (Huston et al., 1997) antibodies were added to initiate capture of PDE4B complexes. Levels of immunoprecipitated PDE4B and associated DISC1 were determined by probing immunoblots of the samples with sheep polyclonal pan-PDE4B antibody (1:40,000) or rabbit polyclonal antibody ZMD.488 (1:500; Invitrogen).

PDE4B Activity
The activity of PDE4B selectively immunoprecipitated from whole-brain lysates was assayed as described (Marchmont and Houslay, 1980).

Further details of all experimental procedures are provided in the Supplemental Data.

Supplemental Data
The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/54/3/387/DC1/.

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