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ORIGINAL ARTICLE

Differential effects of prenatal and postnatal expressions of mutant human DISC1 on neurobehavioral phenotypes in transgenic mice: evidence for neurodevelopmental origin of major psychiatric disorders

Y Ayhan[1], B Abazyan[1], J Nomura[1], R Kim[1], B Ladenheim[2], IN Krasnova[2], A Sawa[3,4,5], RL Margolis[1,4,6], JL Cadet[2], S Mori[7], MW Vogel[8], CA Ross[1,3,4,6,9] and MV Pletnikov[1,3,4]

[1]*Division of Neurobiology, Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [2]*Molecular Neuropsychiatry Branch, NIDA, NIH, DHHS, Baltimore, MD, USA;* [3]*Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [4]*Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [5]*Program in Molecular Psychiatry, Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [6]*Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [7]*Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [8]*Maryland Psychiatric Research Center, University of Maryland, Baltimore, MD, USA and* [9]*Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Strong genetic evidence implicates mutations and polymorphisms in the gene Disrupted-In-Schizophrenia-1 (*DISC1*) as risk factors for both schizophrenia and mood disorders. Recent studies have shown that *DISC1* has important functions in both brain development and adult brain function. We have described earlier a transgenic mouse model of inducible expression of mutant human DISC1 (hDISC1) that acts in a dominant-negative manner to induce the marked neurobehavioral abnormalities. To gain insight into the roles of DISC1 at various stages of neurodevelopment, we examined the effects of mutant hDISC1 expressed during (1) only prenatal period, (2) only postnatal period, or (3) both periods. All periods of expression similarly led to decreased levels of cortical dopamine (DA) and fewer parvalbumin-positive neurons in the cortex. Combined prenatal and postnatal expression produced increased aggression and enhanced response to psychostimulants in male mice along with increased linear density of dendritic spines on neurons of the dentate gyrus of the hippocampus, and lower levels of endogenous DISC1 and LIS1. Prenatal expression only resulted in smaller brain volume, whereas selective postnatal expression gave rise to decreased social behavior in male mice and depression-like responses in female mice as well as enlarged lateral ventricles and decreased DA content in the hippocampus of female mice, and decreased level of endogenous DISC1. Our data show that mutant hDISC1 exerts differential effects on neurobehavioral phenotypes, depending on the stage of development at which the protein is expressed. The multiple and diverse abnormalities detected in mutant DISC1 mice are reminiscent of findings in major mental diseases.
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Introduction

Schizophrenia and mood disorders are believed to arise in part from subtle defects in the development of the cerebral cortex, hippocampus, and other forebrain structures.[1–4] Symptoms of schizophrenia generally appear in late adolescence and early adulthood.[5,6] However, some key pathogenic processes may begin much earlier, as proposed by the neurodevelopmental hypothesis of schizophrenia[4] that postulates that both prenatal and/or early postnatal abnormalities can contribute to disease development.[6–8] Genetic studies have identified several promising candidate genes, such as Disrupted-In Schizophrenia-1 (*DISC1*), neuregulin-1, and dysbindin[9–11] that have been implicated in neurogenesis, neuronal migration, dendrite maturation, and synaptogenesis.[10,12] However, the functions of these candidate genes and their mutations across various stages of neurodevelopment remain poorly understood.

Correspondence: Dr MV Pletnikov, Division of Neurobiology, Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 600 North Wolfe Street, CMSC 8-121, Baltimore, MD 21287, USA.
E-mail: mpletnik@jhmi.edu
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In a large Scottish family a balanced (1:11) (q42.1; q14.3) translocation co-segregates with schizophrenia and mood disorders (LOD scores = 4–7). On chromosome 1, the translocation disrupts two genes, named *DISC1* and *DISC2*.[13,14] *DISC1* or the region of the *DISC1* locus has been implicated in schizophrenia and mood disorders in a number of subsequent genetic analyses, indicating that *DISC1* may be relevant to major mental diseases even in individuals who do not carry the t(1;11) translocation.[15–19]

The DISC1 protein consists of an N-terminal head domain and a long helical C-terminal tail domain[14] and acts as a scaffold protein, with multiple motifs mediating binding to several proteins and facilitating formation of protein complexes.[20–23] The available data have collectively implicated DISC1 in different neurodevelopmental processes, some of which probably extend into adulthood.[18,24–27]

The effect of the translocation may result in *DISC1* haploinsufficiency based on decreased expression of full-length *DISC1* transcript and the failure to detect mutant DISC1 in lymphoblastoid cell lines derived from the patients.[28] However, the available data do not completely rule out the production of mutant DISC protein because lymphoblast expression may not mirror brain expression and available antibodies may not be sufficient to detect mutant DISC1 protein.[29] If a truncated DISC1 protein is expressed in individuals with the translocation, such a protein may have a dominant-negative effect, leading to altered levels and/or distribution of wild-type DISC1 and its binding partners.[30–32] Either dominant-negative or haploinsufficiency mechanisms could similarly perturb DISC1–interacting proteins complexes, resulting in loss of function of DISC1.[18,33] Thus, studying effects of mutant DISC1 on neurodevelopment can provide valuable mechanistic insights into the pathogenesis of psychiatric disorders.

Although abnormal neurodevelopment during pregnancy has been linked to schizophrenia and related psychiatric conditions,[5,7,34] the functions of *DISC1* during prenatal and postnatal periods remain poorly understood. One study has compared early postnatal vs adult effects of inducible expression of a C-terminus fragment of *DISC1* and found that transient expression of this fragment on postnatal day (PND) 7 but not during adulthood produced the distinct morphological and behavioral abnormalities in adult mice.[35] This report was the first to indicate that the neurobehavioral effects of perturbation of DISC1 functions may be time dependent. However, only the effects of transient postnatal expression have been evaluated, and the possible prenatal contribution of mutant DISC1 remains unanswered. Thus, using our mouse model of inducible expression of mutant human DISC1 (hDISC1), we compared the effects of mutant hDISC1 during prenatal, postnatal, or both prenatal and postnatal periods. We evaluated behavioral, pharmacological, biochemical, and morphological alterations in mice in a set of tests relevant to schizophrenia and mood disorders.

Our results show that distinct effects of mutant hDISC1 are dependent on when during neurodevelopment the protein is expressed, consistent with multiple functions of normal DISC1. Given the potential etiologic role of DISC1 in major mental illness, these findings have implications for a better understanding of the relationship between abnormal neurodevelopmental and mental diseases such as schizophrenia and mood disorders.

Materials and methods

Generation of experimental groups

Our mouse model of inducible expression of mutant hDISC1 is based on the Tet-off system (Figure 1a) as has been described earlier.[31] Double-transgenic mice expressed mutant hDISC1 as early as embryonic day 15 (E15), with a gradual decline in expression toward adulthood (Figures 1b and c). This study was conducted using line 1001, which has a high level of expression of the mutant protein.[31] We retained the original mixed background (B6; SJL; CBA) of this line to evaluate how different periods of expression of mutant hDISC1 would affect the neurobehavioral abnormalities described earlier in these mice. Expression of mutant hDISC1 was regulated by Dox-containing food (200 mg kg^{-1} of Dox, Bio-Serv, Frenchtown, NJ, USA). Approximately 5–7 days were sufficient for shutting down or restoring expression of mutant hDISC1 by adding or withdrawing Dox food, respectively (Figure 1d). To study how the neurobehavioral effects of mutant hDISC1 are dependent on the time when expression takes place, we generated four experimental groups of mice with the same genetic make-up but different periods of expression: (1) mice with combined postnatal and prenatal expression (the Pre + Post group); (2) mice with prenatal expression only (the Pre group); (3) mice with postnatal expression only (the Post group); and (4) mice that did not express mutant hDISC1 (the NO group) (Figure 1e). Mice of the Pre + Post group (prenatal and postnatal expression) were conceived, raised, and maintained throughout the entire life on regular food to provide continuous expression of mutant hDISC1. Mice of the Pre group (prenatal expression only) were conceived and raised on regular food until embryonic day 17 (E17). At E17, we started giving pregnant mice Dox-containing food that was continuously provided to the offspring after birth and until they were killed. Mice of the Post group (postnatal expression only) were conceived by parents on Dox food that was continuously provided to pregnant dams until embryonic day 12 (E12). At E12, Dox food was switched to regular food, and dams and their offspring were maintained on regular food until they were killed. Mice of the NO group (no expression) were conceived, raised, and maintained throughout the life on Dox food. For all groups, pups were weaned on PND 21, genotyped, and housed in sex-matched groups of five in standard mouse cages in accordance with Johns Hopkins University Animal Care and Use Committee guidelines.

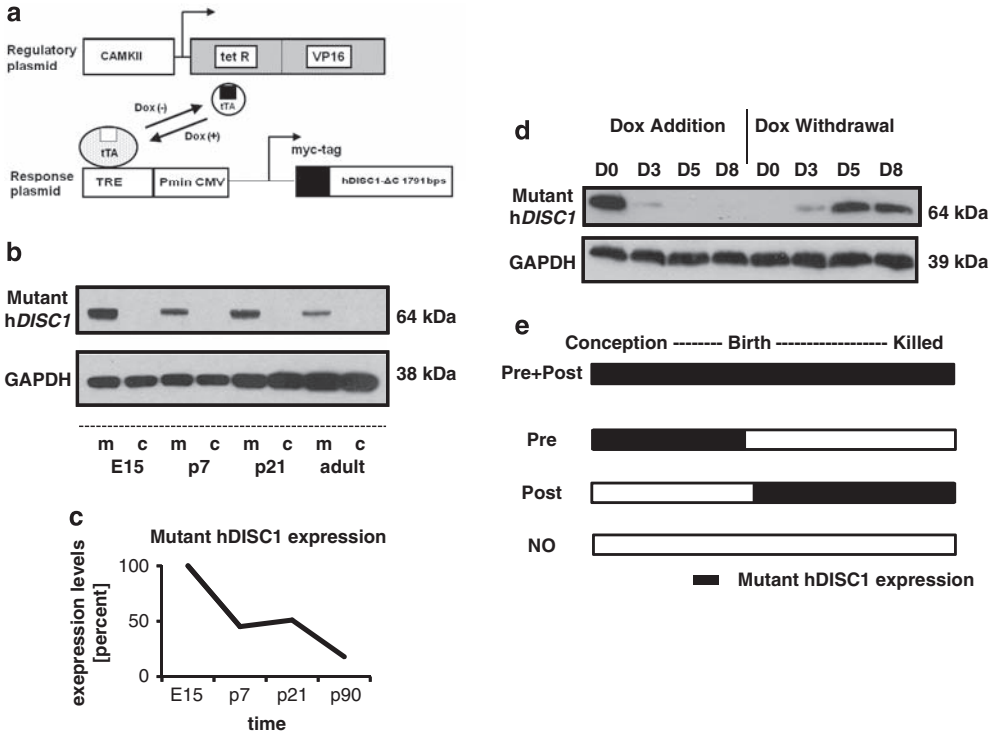

Figure 1 Inducible expression of mutant h*DISC1*. (**a**) The scheme of the Tet-off system used to generate transgenic mice. (**b**, **c**) Expression of mutant h*DISC1* in the cortex at embryonic day 15 (E15, PND 7 (p7), PND 21 (p21), and adulthood (adult) in double-transgenic mutant DISC1 mice (mutant) or single transgenic tTA mice (control). Mutant h*DISC1* was visualized with anti-myc antibody (1:1000) and detected as a 64 kDa band. Anti-glyceraldehyde-3-phosphate dehydrogenase antibody (1:10000) was used as loading control. (**d**) Regulation of expression with Dox food. Adding Dox food to or withdrawing it from mouse diet resulted in shutting down or restoring, respectively, expression of mutant hD*ISC1* within 5–7 days; day 0—the positive control sample before adding (expression) or before withdrawing (no expression) Dox food. (**e**) The experimental groups used in the study. The Pre + Post group had no exposure to the Dox food and expressed mutant h*DISC1* during prenatal and postnatal periods; the Pre group was given Dox after E17 and expressed mutant hD*ISC1* during the prenatal period only; the Post group was given Dox until E12 and expressed mutant hD*ISC1* during the postnatal period only; the NO group was given Dox food all the time and had no expression of mutant h*DISC1* throughout the entire life. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Western blot assays

For western blot assays, mice were euthanized at E15, PND 7, 21, or as adults on completion of behavioral tests (~7–9 months of age) to evaluate expression of mutant hDISC1 protein. Brains were quickly removed and frontal cortex was isolated on ice-cold phosphate-buffered saline and frozen on dry ice and kept at −80 °C until used. These samples were assayed for expression of mutant hDISC1, endogenous mouse DISC1, LIS1, and NDEL1 as described earlier.[31] Membranes were probed with anti-*myc* antibody (1:1000) to assess expression of mutant hDISC1 tagged with *myc,*[31] anti-mouse DISC1 antibody for endogenous DISC1 (1:500),[31] anti-LIS1 antibody (1:1000), or anti-NDEL1 (1:1000) for overnight at 4 °C. Antibodies used were monoclonal to myc (Santa Cruz Biotechnology Inc., CA, USA), monoclonal to LIS1 (Sigma, St Louis, MO, USA), rabbit polyclonal to NDEL1 (Abcam, UK) followed by corresponding peroxidase-conjugated goat anti-mouse (1:1000, Kierkegaard Perry Labs) or sheep anti-rabbit (1:2500, GE Healthcare) secondary antibodies. The optical density of protein bands on each digitized image was normalized to the optical density of the loading control (glyceraldehyde-3-phosphate dehydrogenase, Cell Signaling Inc., USA, 1:10 000) and then normalized to the optical density of sample from control animals (internal reference control). Normalized values were used for analyses.

Behavioral tests

Behavioral tests were performed in mice of 3–7 months of age. The interval between different behavioral tests was 1 week. The tests were performed in the following order: social interaction test; forced swim test (FST); tail suspension test (TST); and drug-induced activity.

Dyadic male–male interaction in the unfamiliar open field. Our earlier study showed altered social interaction patterns and increased aggression in male mice expressing mutant hDISC1.[31] Therefore, we evaluated how the temporal pattern of mutant hDISC1 expression would affect this phenotype. Male–male interaction was analyzed using the protocol described by Rodriguiz *et al.*[36] Briefly, male

mice were housed individually for 4 days to increase social motivation before testing. On the day of testing, one unfamiliar control mouse was paired with a mutant mouse in an activity chamber (San Diego Instruments Inc., San Diego, CA, USA). The chambers were cleaned prior and between each test with MB-10 solution and wiped dry. Mice were simultaneously placed on opposite sides of a cage that was divided into two sections by a solid cardboard partition. After 5 min acclimatization, the partition was removed and the animals were allowed to freely interact for 10 min. All mouse behaviors were videotaped and subsequently scored for sniffing, following, paws on head, attacks, bites, and tail rattling. Each control male mouse was used only once for paring with each mutant hDISC1 mouse.

FST and TST. FST was performed in a large plastic cylinder filled with water as described earlier.[37] The mouse's behaviors in the water tank were videotaped for 15 min daily for 2 consecutive days. Latency to floating and total immobility during the last 5 min on each day were scored.[38] TST was performed in the test chamber (Med Associates, IN, USA). The mouse was suspended by its tail with the hook connected to the movement sensors. The mouse's behaviors were scored for 6 min and the latency to immobility and time of total immobility during the last4 min of testing were analyzed.

Drug-induced locomotion. Drug-induced activity in the open field was assessed over a 60 min period using activity chambers with infrared beams (San Diego Instruments Inc.) as described earlier.[31] First, animals were habituated to the chambers for 30 min, and MK-801 (Sigma-Aldrich, UK) or D-amphetamine (Sigma-Aldrich) was administered intraperitoneally in a dose of 0.3 or 1.0 mg kg^{-1}, respectively. Horizontal and vertical activities, stereotypic activities, and time spent in the center or along the walls (thigmotaxis) of the chamber were automatically recorded.

High-performance liquid chromatography with electrochemical detection

On completion of behavioral experiments, animals were quickly euthanized by cervical dislocation and their brains were isolated and dissected into olfactory bulbs, frontal cortex, hippocampus, striatum, and cerebellum. Tissue content of norepinephrine, dopamine (DA), and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovalinic acid, and 5-hydroxytriptamine and its metabolite 5-hydroxyindoleacetic acid were assayed in the brain regions of male ($n = 5$) and female ($n = 5$) mice. Concentrations of monoamines were measured by high-performance liquid chromatography with electrochemical detection.[39] Monoamine peaks were identified by retention times. Data are expressed as ratios of values in each group in relation to the averaged values in the NO group.

Histopathological and immunohistochemical assays

On completion of behavioral experiments, mice were deeply anesthetized with Euthasol (Diamond Animal Health Inc., Des Moines, IA, USA) and perfused with ice-cold phosphate-buffered saline (pH = 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were removed, postfixed for 4 h, cryoprotected in 30% sucrose in phosphate-buffered saline, and slowly frozen at −20 °C in 2-butane and stored at −80 °C. Brains were sagittally cut in 40 μm sections and stored in the cryoprotection medium at −20 °C until staining. Some sections were stained with cresyl violet for stereological assessment. To evaluate expression of interneuronal markers, brain sections were stained with mouse anti-parvalbumin (PV) (Abcam, MA, USA) (1:100) or rabbit anti-calretinin (CR) antibodies (Abcam) (1:100). Reagents from the ABC kit were used for the blocking solution and the secondary antibody according to manufacturer's recommendations. PV- or CR-positive cells throughout the cortex were counted by an observer blinded to the experimental group with a bright field light microscope. The fronto-temporal cortex was defined as the area starting from the rhinal fissure and continuing through the motor cortex. The temporo-parietal region was defined as the cortical area between somato-sensorial cortices. The parieto-occipital area was defined as the region beginning at the parietal association areas and included the posterior parts of the cortex (plates 110–122 from Franklin and Keith's mouse brain atlas).[40] Sections from the same levels were used ($n = 4$–5 mice per group; five adjacent sections per mouse).[40] Numbers of cells were averaged across all sections for each mouse for the selected area and were used for statistical analyses. Images were captured by Olympus microscope with a Nikon digital camera (DX M 1200) and processed with Nikon ACT-1 software.

Magnetic resonance imaging

On completion of behavioral tests, live mice anesthetized by isoflurane were imaged with a 9.4 T nuclear magnetic resonance scanner (Bruker Biospin, Billerica, MA, USA). Fast-spin echo sequence was used for T2 weighted imaging with following parameters: TR = 4.7 s and effective TE = 22.4 ms, echo train length = 4. Multiple slice 2D images were acquired with in-plane imaging matrix 192 168 and field of view $20 \times 20\,mm^2$. Slice thickness were 0.4 mm without gap between slices. Slice number was 60, covering the whole brain. The imaging resolution was $0.1 \times 0.12 \times 0.4\,mm^3$. With six signal averaging, the scanning time was 40 min as described earlier.[31]

Volumetric measurements

Approximately 15–18 sections were systematically (for example, every 4th) selected from a random start to cover the entire region in question. We used one half of each brain for this assay as described earlier.[41] The measurements were performed using an Olympus microscope with a computer driven X,Y,Z-stage

controller (ASI, Eugene, OR, USA). The total volume of the neocortex was estimated with Cavalieri point counting using the software Stereologer (SPA, Alexandria, VA, USA). The volume of each point was optimized to maximize the efficiency of the process while maintaining the co-efficient of error at 0.1 or less.[41]

Golgi staining-based analysis
A modified, rapid Golgi staining was performed according to the manufacturer's protocol (FD Neuro-Technologies, Germantown, MD, USA). On completion of Golgi–Cox staining procedure, we evaluated the linear spine density on secondary and tertiary branches of basilar dendrites of pyramidal neurons in the temporo-parietal cortex, pyramidal neurons of the CA1 region, and granule cells of the dentate gyrus of the hippocampus and Purkinje cells of the cerebellum. A trained investigator blinded to the group's status performed neurons selection and tracing. Pyramidal neurons were identified by their specific triangular shape of the soma and their apical extensions toward pial surface. An Olympus microscope was used to trace each neuron using Neurozoom (San Diego, CA, USA). For spine density measurement, one terminal dendrite from the second and third order tip of each selected neuron was used to count spines using a 100 × objective. Five neurons per section and five sections per mouse (four mice per group) were used to count the linear spine density. The results are presented as adjusted values relative to the NO group. Images of Golgi staining were captured by Olympus microscope with a Nikon digital camera (DX M 1200) and processed with Nikon ACT-1 software.

Statistical analysis
The effects of mutant h*DISC1* on mouse behaviors, regional monoamine levels, and neuroanatomical measures were evaluated with a mixed model ANOVA with the group, sex, and time of testing (if applicable) as independent variables. Significant effects were explored further with lower levels ANOVAs and/or *post hoc* comparisons. $P<0.05$ was used for the significance level.

Results

Regulation of mutant hDISC1 expression
As expected, expression of mutant hDISC1 was present in the Pre + Post and Post groups and was absent in the Pre and NO groups when assessed at PND 120 (Supplementary Figure 1).

The behavioral effects of prenatal mutant hDISC1 expression

Social interactions. We have reported earlier abnormal social behaviors in male mutant hDISC1 mice.[31] Here, we found that male mice of the Pre + Post group and Post groups spent significantly less time in non-aggressive social interaction with their partners compared with the NO group, all Ps <0.05 (planned *t*-test). No differences in non-aggressive social interaction were found between the Pre and NO groups (Figure 2a). Male mice of the Pre + Post group showed significantly more aggressive attacks than animals of the NO group, $P<0.05$. No significant differences in aggression were found between other groups (Figure 2b).

FST and TST. FST and TST are widely used to evaluate depression-like behaviors in rodents.[42,43] Earlier studies have reported aberrant responses in other *DISC1* mouse models in these tests,[35,37,44,45] which is consistent with the human data on association between *DISC1* polymorphism and depression-related abnormalities.[46–48] Thus, we evaluated these behaviors in this study. Female mice of the Pre + Post group spent significantly more time in immobility compared with female mice of the NO group during the last 4 min in TST, $F(3, 35)=4.212$, $P=0.012$; Pre + Post vs NO, $P<0.05$, whereas there were no differences in this measure between other groups (Figure 2c). In FST, female mice of the Post group spent significantly more time in immobility compared with mice of the NO group during the last 5 min on the second day of testing, $F(3, 18)=4.993$, $P=0.01$, Post vs NO, $P<0.05$. No differences in FST were found between female mice of other groups (Figure 2d). In addition, expression of mutant hDISC1 had no effects on these behaviors in male mice of either group (data not shown).

The effects of MK-801 and D*-amphetamine.* Amphetamines and N-methyl-D-aspartic acid antagonists can produce psychosis-like behaviors in humans and increase locomotor activity in rodents.[49–51] These compounds have also been used to analyze dopaminergic and glutamatergic neurotransmission in various models of schizophrenia.[52–54] We therefore evaluated the effects of MK-801, a non-competitive N-methyl-D-aspartic acid antagonist, and D-amphetamine, an indirect DA agonist, on locomotor activity of mutant h*DISC1* mice. The relatively low doses were selected to better identify potential difference in response to stimulants.[55,56] MK-801 injections ($0.3\,mg\,kg^{-1}$, intraperitoneally) resulted in significantly greater total locomotor activity in male mice of the Pre + Post group compared with that in male mice of three other groups (the group effect, $F(3352)=3.723$, $P=0.021$; Pre + Post vs NO and Pre + Post vs Post, all Ps <0.05) (Figures 2e and f). No differences in MK-801-induced activity were seen between the groups of female mice (data not shown). Administration of D-amphetamine ($1\,mg\,kg^{-1}$, intraperitoneally) significantly increased locomotor activity in male mice of the Pre + Post group compared with the other groups (the group by time interaction, $F(33, 418)=1.872$, $P=0.03$) (Figure 2g). Total locomotor activity for the first 15 min post injection was significantly greater in the Pre + Post group than the Pre or NO group (the group effect for

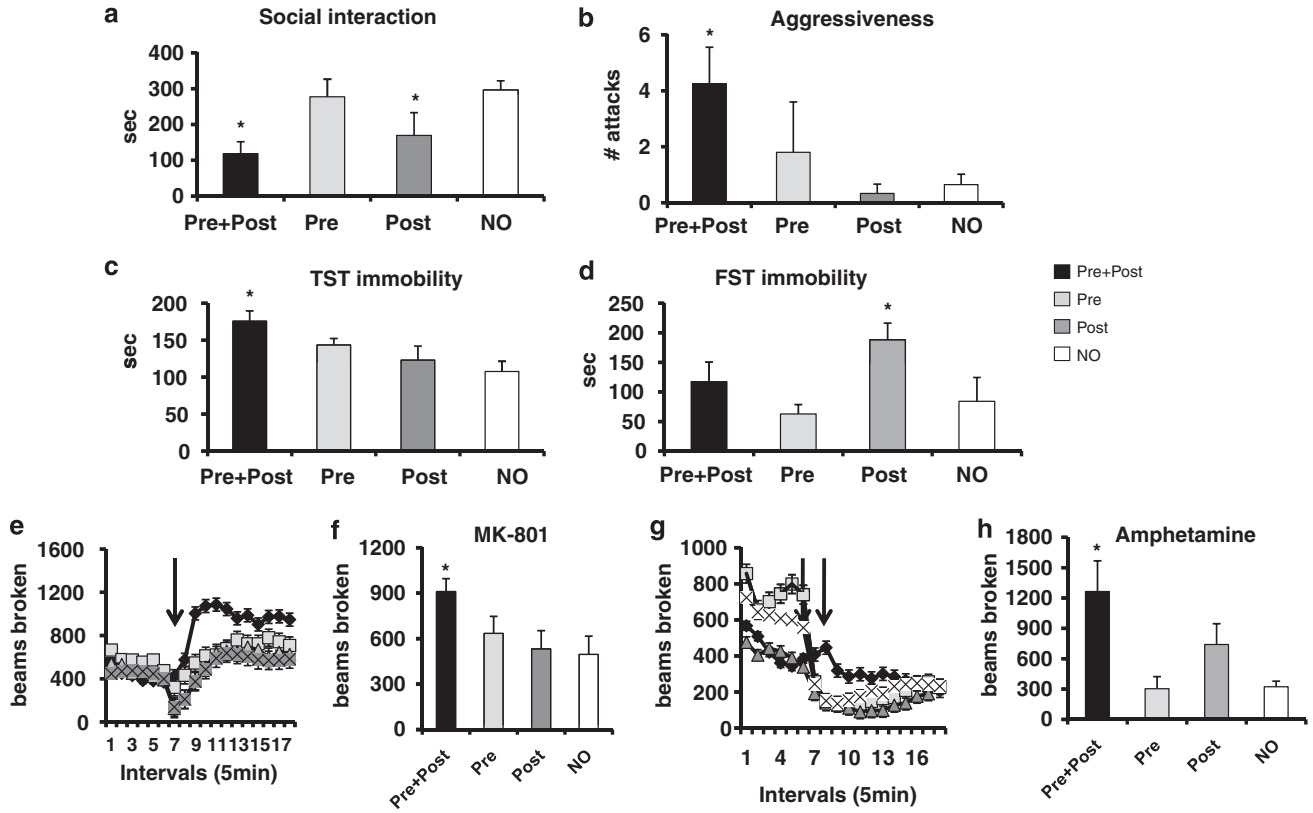

Figure 2 Time-dependent behavioral effects of mutant h*DISC1*. (**a**) Decreased non-aggressive social interaction in the Pre + Post and Post groups compared with the NO group, *denotes $P < 0.05$ vs the NO group; $n = 7$–8 male mice per group. (**b**) Aggressive attacks in mice. Note significantly more attacks in the Pre + Post group compared to the NO group, *denotes $P < 0.05$ vs the NO group, $n = 7$–8 male mice per group. (**c**) Time of immobility in tail suspension test (TST). Note increased time of immobility in mice of the Pre + Post group compared to the NO group, *denotes $P < 0.05$ vs the NO group; $n = 6$ female mice per group. (**d**) Time of immobility in forced swim test (FST). Note increased time of immobility in the Post group compared to the NO group, *denotes $P < 0.05$ vs the NO group, $n = 6$ female mice per group. (**e**) MK-801-induced locomotor activity. Note greater drug-induced activity in the Pre + Post group compared to other groups, $n = 8$–12 male mice per group; the arrow points to the time of injection. (**f**) The effect of MK-801 on the total activity over one hour. The mean values of total locomotor activity over 1 h are presented. Note the significantly increased locomotor activity in the Pre + Post group compared to the NO group, *denotes $P < 0.05$ vs the NO group. (**g**) D-amphetamine-induced locomotor activity. Note significantly increased activity in the Pre + Post group compared to other groups, $n = 6$–8 male mice per group; the arrow points to the time of injection. (**h**) The effect of amphetamine injection on total locomotor activity during the first 15 min post injection. The mean values of total locomotor activity over 15 min are presented. Note greater drug-induced activity in the Pre + Post group vs other groups, *denotes $P < 0.05$ vs the NO or Pre groups.

the first 15 min, $F(3, 38) = 5.170$, $P = 0.04$; Pre + Post vs NO and Pre + Post vs Pre, all Ps < 0.05). (Figure 2h). Similar to the results with MK-801, no significant differences in amphetamine-induced activity were found between the groups of female mice (data not shown). No significant differences in other measures in this test were found (data not shown).

Tissue content of monoamines and their metabolites

Alterations in monoamine neurotransmission have been associated with schizophrenia and mood disorders.[54,57] There are no available data for possible monoamines perturbations associated with expression of mutant hDISC1 in mice. Thus, we evaluated the tissue content of norepinephrine, DA, 5-hydroxytriptamine and their metabolites, DOPAC, and 5-hydroxyindoleacetic acid in mutant hDISC1 mice. Compared with the values of male mice of the NO group, there was a significant decline in levels of DA and DOPAC in frontal cortex of male mice of all other groups, (Figure 3a) (the main group effect, $H = 9.81$, $P = 0.02$, and $F(3, 16) = 3.65$, $P = 0.035$ for DA and DOPAC, respectively). *Post hoc* comparisons showed the significant differences in DA content between the NO group and each of the other groups, all Ps < 0.05. There were significant differences in DOPAC content between the Pre + Post vs NO groups and the Post vs NO groups, all Ps < 0.05 (Figure 3a). No significant differences were found among the groups in 5-hydroxyindoleacetic acid/5-hydroxytriptamine, DOPAC/DA, or homovalinic acid + DOPAC/DA ratios in cortical samples (Figure 3b). For female mice, we found a significant decrease in DA content in the hippocampus of the Post group compared to NO or Pre groups (Figure 3c), $F(3, 713) = 13.15$, $P = 0.035$; Post vs NO and Post vs Pre, all Ps < 0.05. No other

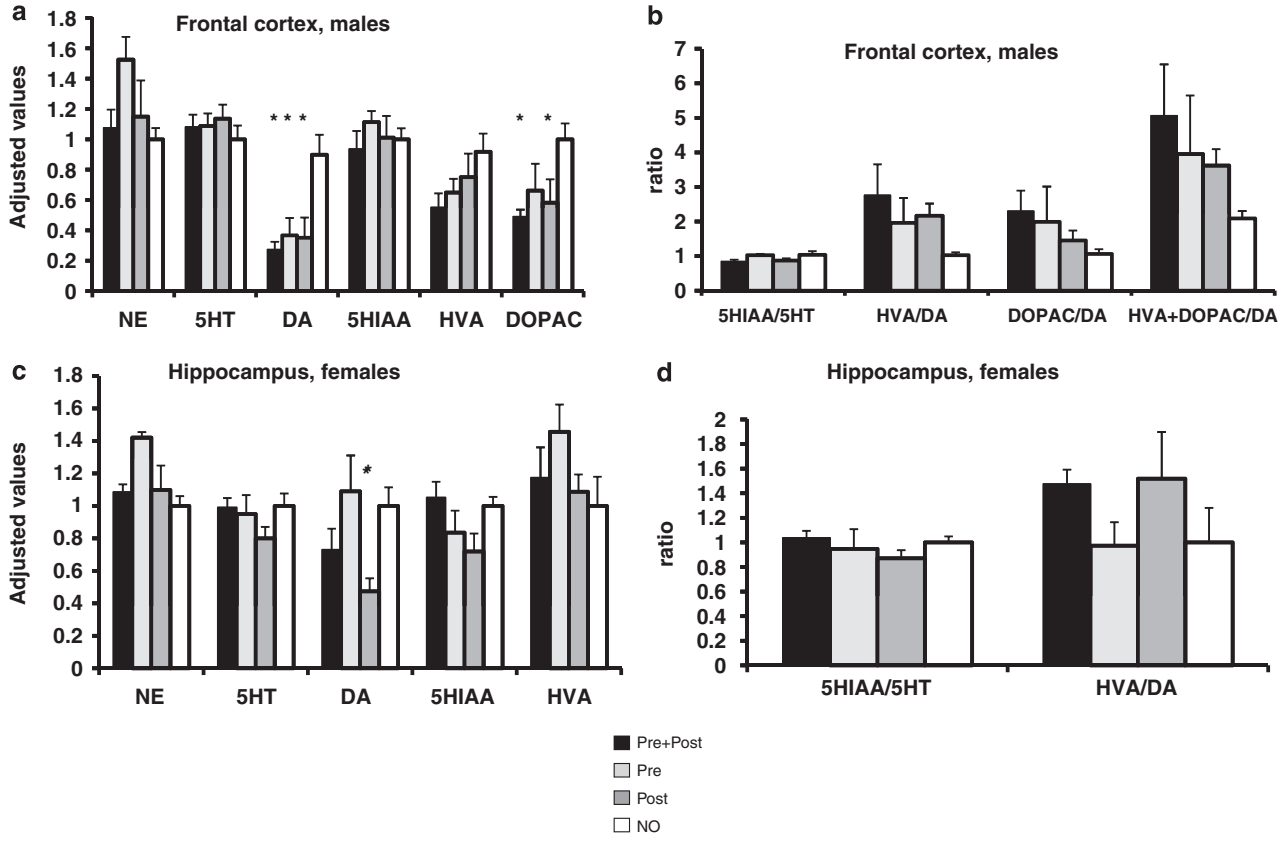

Figure 3 Regional alterations in levels of monoamines. (**a**) A significant decrease in tissue content of dopamine (DA) and 3,4 dihydroxy-phenylacetic acid (DOPAC) in cortex of male mice of either group compared to male mice of the NO group, *denotes $P<0.05$ vs the NO group, $n=5$–6. (**b**) No significant changes in monoamine turnover in frontal cortex of male mice. (**c**) Tissue content of DA was significantly decreased in the hippocampus of female mice of the Post group compared to female mice of the Pre or NO groups, *denotes $P<0.05$ vs the Pre or NO groups, $n=4$–6. (**d**) No significant changes in monoamine turnover in the hippocampus of female mice.

significant alterations in content of monoamines or their metabolites were found in the brain regions assayed (Figure 3d; Supplementary Figure 2).

Expression of markers of γ-aminobutyric acid (GABA)-ergic neurons

Reduced PV immunoreactivity has been found in postmortem schizophrenia samples, suggesting a role for altered GABA-ergic signaling in schizophrenia.[58,59] Expression of another calcium-binding protein, CR, on the other hand, does not seem to be consistently reduced in schizophrenia.[60] Earlier studies with other mutant hDISC1 transgenic mice have shown a reduction in the numbers of PV-positive neurons in the cortex.[37,45] We evaluated numbers of PV and CR positively stained (+) cells in the frontal, parietal, and occipital areas of the cortex (Figures 4a–h). Numbers of cortical PV+ cells were significantly reduced in all *DISC1* expressing groups compared with the NO group (the group effect for total cortex, $H=25.357$, df = 3, $P<0.001$, the Pre vs NO, the Post vs NO, and the Pre + Post vs NO group, all Ps <0.05; for Ftcx –F(3157) = 7.65, $P<0.001$, the Pre vs NO, the Post vs NO, and the Pre + Post vs NO group, all Ps <0.05; Tpcx $H=10.22$, $P=0.02$ the Pre + Post vs NO group, $P<0.05$; Pocx $-H=11.894$, $P=0.008$, the Post vs NO and the Pre + Post vs NO group, all Ps <0.05) (Figure 4i). Numbers of CR+ cells were not significantly changed although there was a trend to a decrease in numbers of CR+ cells in the frontal cortex (the main effect for total cortex $F(3, 40)=1.120$, $P=0.352$; Ftcx $-F(3, 47)=2.367$, $P=0.08$; Tpcx $-F(3, 48)=2.150$, $P=0.1$; Pocx $-F(3, 45)=0.328$, $P=0.8$) (Figure 4j).

Volumetric assays

Magnetic resonance imaging analyses. Lateral ventricle enlargement is one the most consistent abnormalities of the brain of patients with schizophrenia.[5,61,62] Earlier studies with *DISC1* mouse models, including our model, have found enlarged ventricles in adult mice.[31,44,45] Thus, we evaluated the effects of prenatal and postnatal expressions of mutant hDISC1 on brain and ventricle volumes (Figures 5a–d). We did not find significant effects of gender on both measures, ($F(3, 24)=1.687$, $P>0.05$ for lateral ventricles, and $F(3, 24)=1.145$; $P>0.05$ for brain volume (Supplementary Figure 3). Thus, we combined the data for male and female mice for all subsequent

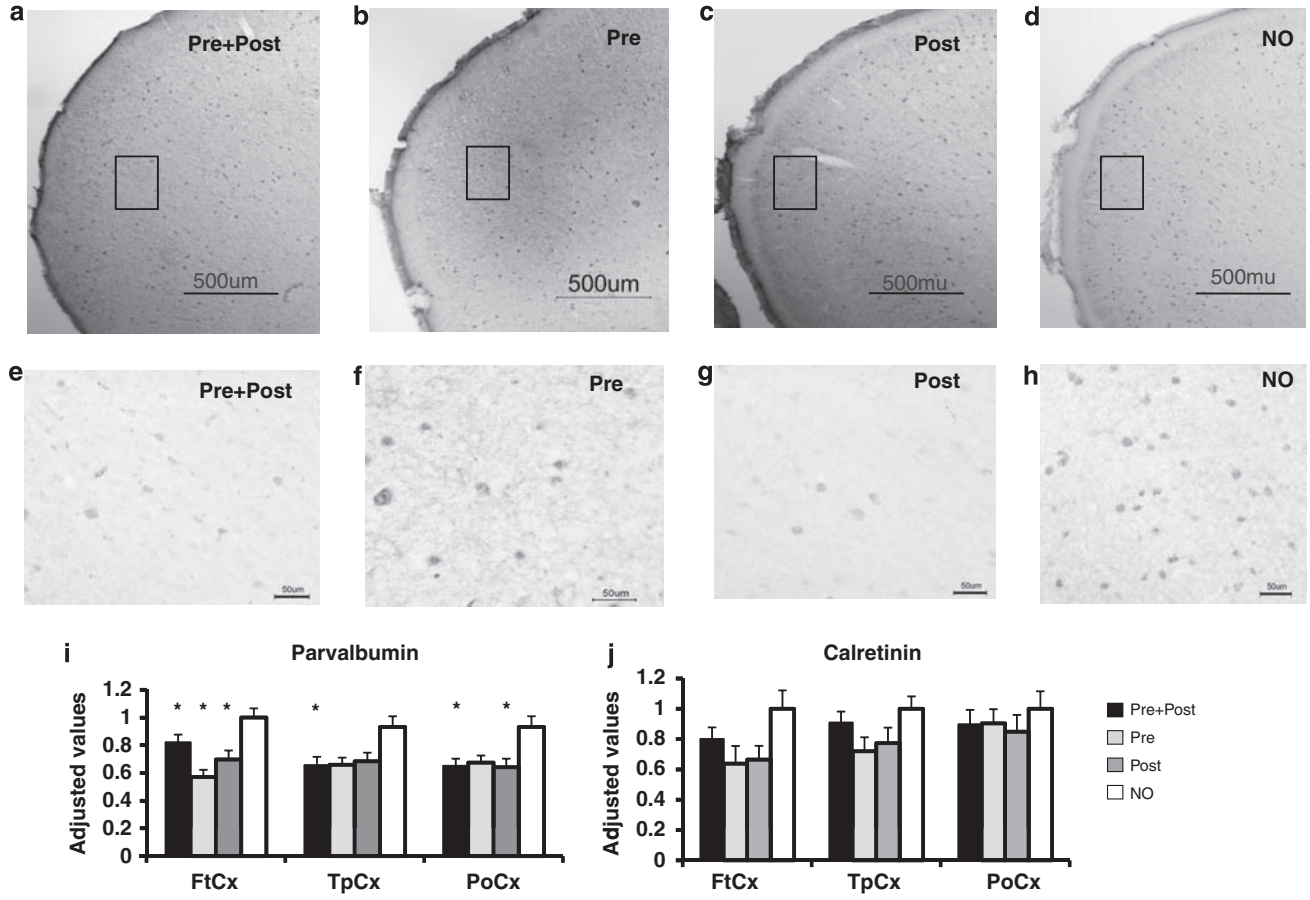

Figure 4 Decreased numbers of parvalbumin (PV)-positive interneurons in mutant DISC1 transgenic mice. PV-positive cells in frontal cortical areas of the Pre + Post (**a**, **e**), Pre (**b**, **f**), Post (**c**, **g**), and NO (**d**, **h**) groups; '**a–d**' panels are low magnification images, scale bar, 500 μm; '**e–h**' panels are high magnification images, scale bar, 50 μm. A quantitative analysis showed a significant decrease in numbers of PV-positive cells throughout the entire cortex in the Pre + Post, Pre, and Post groups compared to the NO group (**i**). The significant decrease was found in fronto-temporal (Ftcx), temporo-parietal (Tpcx), and parieto-occipital (Pocx) cortices; $n = 4$–6 mice per group, *denotes $P < 0.05$ vs the NO group. No significant changes in numbers of calretinin (CR)-positive cells were found, $n = 4$–6 mice per group (**j**).

analyses. Lateral ventricles were significantly larger in the Pre + Post or Post groups than in the Pre or the NO groups, $F(3, 24) = 3.903$, $P = 0.01$; all Ps < 0.05 (Figure 5e). Total brain volume was significantly smaller in the Pre compared with the Post or NO groups, $F(3, 24) = 7.07$, $P = 0.001$; the Pre vs Post group and the Pre vs NO group, all Ps < 0.05 (Figure 5f).

Stereological analyses. Decreased volumes of cortex and other brain regions have been observed in schizophrenia[10,63,64] and *DISC1* has been directly implicated in cortical development.[30] Thus, we assessed the effects of prenatal vs postnatal expression of mutant h*DISC1* on cortical volumes. We found smaller cortical volumes in the Pre + Post and Post groups compared to the NO group, $F(3, 21) = 4.260$, $P = 0.017$; Pre + Post vs NO and Post vs NO group, Ps < 0.05. (Figure 5g).

Dendritic spine density

Alterations in dendritic spine density have been shown in several psychiatric disorders.[65–67] In addition, there are reports of decreased spine density in hippocampal granule cells in the *DISC1* mouse model.[68] We evaluated the linear density of dendritic spines on granule cells of the dentate gyrus of the hippocampus, pyramidal neurons of the CA1 region of the hippocampus, pyramidal neurons of the temporo-parietal cortex, and the Purkinje cells of the cerebellum as an internal control area that does not express mutant hDISC1.[31] We found a significant increase in the linear spine density on dendrites of hippocampal granule cells in the Pre + Post group compared with all other groups, $F(3, 14) = 7.244$, $P = 0.04$; all Ps < 0.05 (Figure 5h). In addition, the linear spine density on dendrites of pyramidal cortical neurons was significantly greater in the Pre group than in the NO group, $F(3, 13) = 4.32$, $P = 0.026$; *post hoc* test, $P < 0.05$ (Figures 5h and i–l).

Expression of DISC1–interacting proteins

Mutant h*DISC1* has been proposed to exert its effects through dominant-negative mechanisms.[30,32] We have found earlier that expression of mutant h*DISC1* was

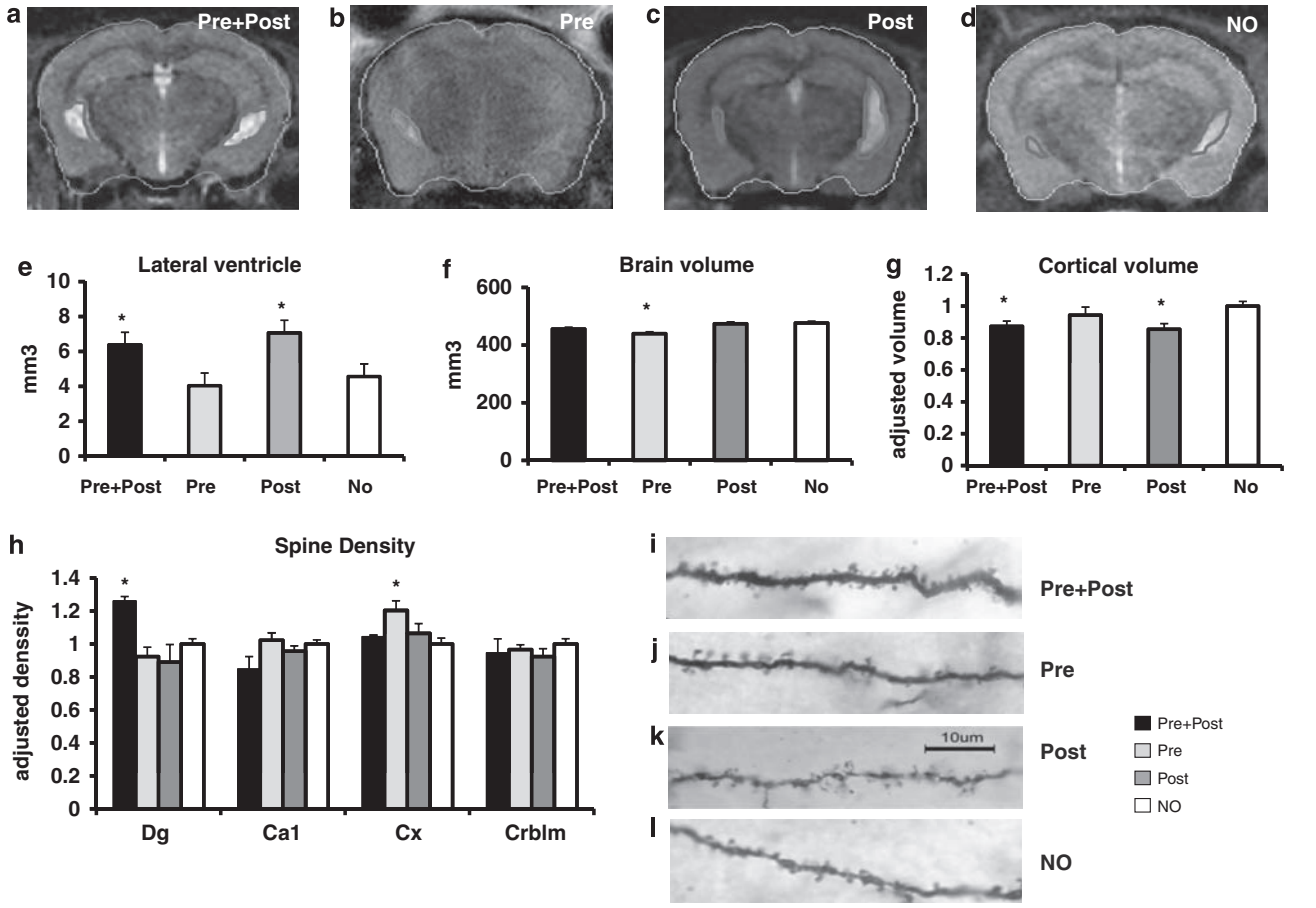

Figure 5 Morphometric analyses of the effects of mutant h*DISC1*. (**a–d**) Representative magnetic resonance imaging coronal images for the Pre + Post (**a**), Pre (**b**), Post (**c**), and NO (**d**) groups. The boundaries of the brain and the lateral ventricles are outlined. (**e**) The significantly increased volumes of the lateral ventricles in the Pre + Post and the Post group compared to the NO group, $n = 8$ mice per group, *denotes $P < 0.05$ vs the NO group. (**f**) The significantly decreased total brain volumes in the Pre group compared to the Post or the NO groups, $n = 8$ mice per group, *denotes $P < 0.05$ vs the Post or NO groups. (**g**) The significantly decreased cortical volumes in the Pre + Post and Post groups compared to the NO group, $n = 8$ mice per group, *denotes $P < 0.05$ vs the NO group. (**h**) A quantitative analyses of the linear spine density on dendrites of granule cells of the dentate gyrus (Dg), CA1area (Ca1) of the hippocampus, pyramidal neurons of the temporal cortical area (Cx), and the Purkinje cells of the cerebellum (Crblm); *denotes $P < 0.05$ vs the other groups; #denotes $P < 0.05$ vs the NO group; $n = 10$–20 neurons per mouse, four mice per group; representative images of dendritic spines from the Pre + Post (**i**), Pre (**j**) and Post (**k**), and NO (**l**) groups, scale bar, 10 μm.

associated with decreased protein levels of endogenous mouse DISC1 and LIS1.[31] Here, we evaluated levels of endogenous DISC1, LIS1, and NDEL1 in mice that expressed mutant hDISC1 during prenatal or postnatal periods. Expression of endogenous DISC1 and its interacting proteins was assayed at PND 7 in cortical samples, the time point when we earlier detected decreased expression of these proteins.[31] We found a significant decrease in protein levels of LIS1 in the Pre + Post group compared with the NO group, $H = 9.502$, df = 3, $P = 0.023$ (Figures 6b and d). In addition, there was a significant decline in level of endogenous mouse DISC1 in the Pre + Post and Post groups compared with the NO group, $F(3, 12) = 4.089$, $P = 0.032$, all Ps < 0.05 (Figures 6a and e). No sex-related differences in expression of mutant, endogenous mouse DISC1 or LIS1 were found (Supplementary Figure 4). No significant differences in protein levels of NDEL1 were detected between groups (data not shown).

Discussion

We have analyzed the phenotypic effects of expression of mutant h*DISC1* during different stages of mouse development. The main findings of the study are summarized in Table 1.

The primary conclusion of our study is that the effects of mutant h*DISC1* are qualitatively and quantitatively different, depending on when during neurodevelopment the protein is expressed. Certain phenotypic changes were present regardless of the time point of expression of mutant h*DISC1*, including fewer PV-positive cells in the cortex and decreased cortical levels of DA. The most profound phenotypic effects were detected after combined

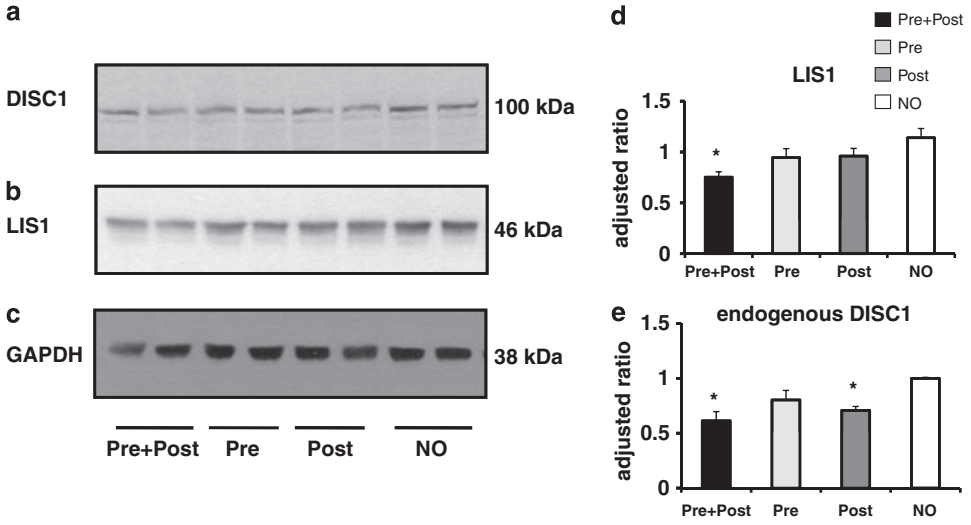

Figure 6 Expression of endogenous DISC1 and LIS1. (**a–c**) Representative blots for endogenous DISC1 (**a**), LIS1 (**b**), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (**c**), cortical samples collected at P7. (**d**) The significantly decreased levels of LIS1 in the Pre + Post group compared to the NO group, $n = 4–5$ samples per group, *denotes $P < 0.05$ vs the NO group. (**e**) The significantly decreased levels of endogenous DISC1 in the Pre + Post and Post group vs the NO group, $n = 4–5$ samples per group, *denotes $P < 0.05$ vs the NO group.

Table 1 Prenatal and postnatal effects of mutant hDISC1

Significant changes vs the NO group (no expression)	*Pre- and postnatal expression, the Pre + Post group*	*Prenatal expression only, the Pre group*	*Postnatal expression only, the Post group*
Decreased non-aggressive social interaction	+	−	+
Increased aggressive behavior	+	−	−
Increased immobility in FST	−	−	+
Increased immobility in TST	+	−	−
Increased sensitivity to psychostimulants	+	−	−
Decreased content of DA in the frontal cortex of male mice	+	+	+
Decreased content of DA in the hippocampus of female mice	−	−	+
Fewer PV-positive cells	+	+	+
Increased volumes of the lateral ventricles	+	−	+
Decreased total volume of the brain	−	+	−
Decreased total volume of the cortex	+	−	+
Increased linear spine density in granule cells of the dentate gyrus of the hippocampus	+	−	−
Increased linear spine density in pyramidal neurons of the cortex	−	+	−

Abbreviations: DA, dopamine; FST, forced swim test; hDISC1, human Disrupted-In-Schizophrenia-1; PV, parvalbumin; TST, tail suspension test.

prenatal and postnatal expression. Specifically, we observed elevated aggression, depression-like responses in female mice, increased responses to stimulants in male mice, and increase density of dendritic spines on neurons of the dentate gyrus of the hippocampus, and decreased levels of endogenous DISC1 and LIS1. Prenatal expression only led to decreased total brain volume, whereas selective postnatal expression of the protein produced attenuated social behavior in male mice, depression-like responses in female mice, enlarged lateral ventricles, decreased DA content in the hippocampus in female mice, and lower protein levels of endogenous DISC1. As mutant hDISC1 seems to perturb functions of endogenous mouse DISC1 through dominant-negative effects, our results indicate that DISC1 may have multiple functions that vary during neurodevelopment.

This study has significantly extended the phenotypic features of our model by adding new assays on behaviors, neurochemistry, and morphology to evaluate the time-dependent effects of mutant h*DISC1*. Consistent with our earlier report, some of the behavioral effects of mutant h*DISC1* were gender specific. Our model is currently the only one to report the gender-specific effects of mutant

h*DISC1*.[31,35,37,45,69] The reasons for these observed differences remain obscure as we found no significant gender-related alterations in the brain morphology or expression of mutant hDISC1, endogenous DISC1, or LIS1 in transgenic mice. Although our results might look congruent with gender-related associations between polymorphisms in *DISC1* and disease frequency or cognitive functions,[15,70,71] this issue awaits further experimental clarifications and replications.

The effects of expression of mutant h*DISC1* on levels of monoamines and their metabolites are consistent with the behavioral and pharmacologic abnormalities in transgenic mice. For example, combined prenatal and postnatal expression of mutant h*DISC1* decreased levels of DA in frontal cortex of male mice, which could contribute to the increased responses to D-amphetamine and MK-801 observed in male mice of the Pre + Post group.[51] The enhanced pharmacologic effects in transgenic mice may indicate the alterations in dopaminergic and glutamatergic systems consistent with findings in patients.[72–74] Reduced levels of DA and to some extent 5-hydroxytriptamine metabolite, 5-hydroxyindoleacetic acid (for example, Figure 3), in the hippocampus of female mice with selective postnatal expression of the protein would seem to be in line with depression-like responses in this group of mice. The results seem to be consistent with a role of *DISC1* in mood disorders where monoamine alterations in the cortex and hippocampus have been shown to contribute to the pathophysiology of affective states.[75–77] However, it should be pointed out that tissue content assays do not provide direct assessment of functional changes in monoamine neurotransmission, and additional investigations based on *in vivo* microdialysis and/or receptor expression and distribution will be necessary to shed more light on perturbations in monoamines in *DISC1* female mice.

PV and CR are markers for inhibitory interneurons.[58] PV immunoreactivity is reduced in the cortex and hippocampus of schizophrenic brains.[58,60,78,79] Lower expression of PV is suggested to alter functional properties of cortical interneurons, leading to dysfunctional activity of cortical pyramidal neurons postulated to contribute schizophrenia pathogenesis.[59,80] We found that both prenatal and postnatal expression of mutant h*DISC1* decreased numbers of PV-positive cells throughout the cortex, consistent with earlier studies of other DISC1 mouse models.[37,45] Despite the similar outcome, mutant h*DISC1* could differently affect maturation of this population of neurons across neurodevelopment. It is conceivable that prenatal expression of mutant h*DISC1* may predominantly disrupt migration interneurons whereas postnatal expression would probably affect final stages of their differentiation.[81,82] Although a decrease in numbers of CR-positive cells in fronto-temporal cortex was not significant, one cannot completely rule out that mutant *DISC1* might produce a more general deficit in GABA-ergic cells. Of note, it has been shown that LIS1 heterozygous mice also show a decrease in GABA-ergic markers, including CR.[82]

Prenatal and postnatal expressions of mutant h*DISC1* differentially affected volumes of the brain and lateral ventricles. We found that mice with prenatal expression of mutant *DISC1* had significantly decreased total brain volumes compared with animals with postnatal expression or mice that did not express mutant *DISC1* at all. Reduced brain volumes in the Pre group appear consistent with earlier reported decreased neuronal proliferation because of *DISC1* knockdown.[83] One can speculate that mutant h*DISC1*, acting through dominant-negative mechanisms, could affect proliferation of neuronal progenitor cells, leading to smaller brain volumes as detected in adult mice.

In contrast, though postnatal expression did not change brain volumes, it was likely responsible for enlargement of the lateral ventricles. The effects of postnatal expression on the lateral ventricles may be in line with the hypothesis that the ventricular pathology in schizophrenia is related to gradual postnatal changes.[84–86] Intriguingly, if postnatal expression of mutant *DISC1* is confirmed to be sufficient to produce enlargement of the lateral ventricles and given that this pathological feature is a consistent one in schizophrenia, this endophenotype could be a promising biological marker for testing novel compounds in developing and adult animals.

We found that combined prenatal and postnatal expression of mutant h*DISC1* led to increased spine density in the dentate gyrus of the hippocampus and selective prenatal expression of the protein was associated with increased spine density in the temporo-parietal cortex. On the one hand, our findings seem discordant with human postmortem reports about decreased linear spine density in frontal cortex, auditory cortex, and subiculum,[66,67,87] and the results reported for a different *DISC1* mouse model.[68] On the other hand, the data presented here are in line with that the effects of *DISC1* knockdown that has produced increased spine density, dendritic branching, arborization, and migration rates in newborn neurons in the dentate gyrus of the adult hippocampus.[88,89] As we did not discriminate immature vs mature spines in this study, one cannot rule out the possibility that increased linear density of protrusions assessed may be related to immature spines that might not function properly. Future studies can address this possibility.

Consistent with the possible etiological roles of *DISC1* in schizophrenia and mood disorders and similar to the effects reported for other *DISC1* models, our transgenic mice exhibited the behavioral alterations reminiscent of aspects of both schizophrenia and mood disorders. The variable multiple effects of variants and mutations of the *DISC1* gene have been proposed to be dependent on the time of expression, interactions with other genes and/or environmental factors.[5,90] In this context, our findings are in line

with diverse clinical manifestations of the translocation mutation in the Scottish family and support the role of *DISC1* as a 'hub' protein with pleiotropic effects at different points across neurodevelopment and in the pathophysiology of different major mental disorders.[5,18,21,91]

One of the possible mechanisms whereby mutant *DISC1* could affect neurodevelopment in our mice is altering functioning of endogenous mouse *DISC1* and its interacting partners. Our earlier study has shown that mutant h*DISC1* can bind to endogenous mouse *DISC1*, producing a reduction in protein levels of endogenous mouse DISC1 and LIS1.[31] This study confirms and extends those data by showing that temporal expression of mutant *DISC1* largely parallels expression of endogenous mouse *DISC1*,[92,93] providing additional evidence that the observed abnormalities in our mice may be due to dominant-negative effects of mutant h*DISC1*. Thus, even if the translocation carriers in the Scottish family do not express the truncated protein, the model of inducible expression of mutant hDISC1 could advance our understanding of altered functions of DISC1 in patients.

This study is limited in comparing the effects of prenatal and postnatal expressions of mutant h*DISC1*. Future research can generate new experimental groups of mice with expression of mutant h*DISC1* across different stages of postnatal life with a more precise correspondence to such periods as early vs late postnatal development, sexual maturation, adulthood, and aging. This line of research can be readily pursued with our model, which allows for regulating when and for how long the protein is expressed.

In conclusion, our results show that the differential neurobehavioral effects of mutant h*DISC1* depend on when across neurodevelopment expression of the protein occurs. These data are consistent with the notion that *DISC1* has different functions across various stages of neurodevelopment and adulthood, which can partially explain diverse *DISC1*-associated pathological manifestations, and potentially provide a model for aspects of major mental diseases.

Conflict of interest

The authors declare no conflict of interest.

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