

Disrupted-In-Schizophrenia-1 Gln31Leu Polymorphism Results in Social Anhedonia Associated with Monoaminergic Imbalance and Reduction of CREB and β -arrestin-1,2 in the Nucleus Accumbens in a Mouse Model of Depression

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Disrupted-in-schizophrenia-1 (DISC1) is associated with mental disorders, including major depression. We previously showed that DISC1-Q31L mutant mice have depression-like behaviors and can therefore be used to study neurobiological mechanisms of depression and antidepressant (AD) medication action. First, we found reduced levels of dopamine, serotonin and norepinephrine in the nucleus accumbens (NAC) of DISC1-Q31L mutants. Next, we assessed social-conditioned place preference as a reward-dependent task and the capacity of distinct ADs to correct impaired social behavior in DISC1-Q31L mice. Bupropion, but not fluoxetine or desipramine, was able to correct deficient social facilitation, social reward, and social novelty in DISC1-Q31L mutants, whereas all three ADs were able to improve social motivation and behavioral despair in DISC1-Q31L mutants. Furthermore, we sought to correlate social anhedonia with molecular and cellular features including dendritic spine density, β -arrestin-1,2, and cAMP-response-element-binding protein (CREB) in the NAC as biomarkers related to depression and the DISC1 pathway. DISC1-Q31L mutants showed reduced levels of β -arrestin-1,2, CREB, and spine density in the NAC, further supporting the construct validity of the genetic model. Bupropion induced the greatest effect on CREB in DISC1-Q31L mutants, whereas all studied ADs corrected the reduced levels of β -arrestin-1,2 and modestly ameliorated deficient spine density in this brain region. Overall, we find neurobiological changes accompanying social anhedonia in the NAC of DISC1-Q31L mutant mice, consistent with a role for DISC1 in regulating social reward as an endophenotype of depression. *Neuropsychopharmacology* (2013) **38**, 423–436; doi:10.1038/npp.2012.197; published online 26 September 2012

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INTRODUCTION

Depression is a common public health problem with a 16% lifetime prevalence and it is one of the leading causes of disability and morbidity worldwide (Treadway and Zald, 2011). Depression, like most other mental disorders, is currently diagnosed using clinical criteria, without reference to etiology or diagnostic biological markers, and at least one third of patients with depression do not improve with existing antidepressant (AD) medication treatments (Rush *et al*, 2006). Our understanding of the pathophysiology and

genetics of depression remain limited (Cryan and Holmes, 2005). Hence, new preclinical animal models to link genetic susceptibility factors with the pathophysiology of depression could help to identify promising molecular targets for new treatments.

Several criteria are used to assess the utility of an animal model, including etiological validity (having the same genetic and environmental factors that cause depression in humans), face validity (the mouse behaviors must be related to symptoms of depression), construct validity (similarity of neurobiological mechanisms as in humans), and predictive validity (ADs should correct abnormal behaviors seen in an animal model) (Cryan and Holmes, 2005). The advent of various techniques for creating mutant mice provides an opportunity to investigate the function of candidate gene and signaling pathways in the pathogenesis of depression. Nearly 80 different mutant mouse strains with phenotypes relevant to depression have been developed (Cryan and

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Holmes, 2005). However, 'none of these discoveries has so far been translated into a new *bona fide* treatment for depression' (Berton and Nestler, 2006).

Disrupted-in-schizophrenia-1 (DISC1) has attracted much attention in the last decade because of its causal role in several psychiatric disorders, including major depression (Bradshaw and Porteous, 2011). Moreover, several genes encoding DISC1 interacting proteins are also independent risk factors for major depression, including dysbindin, phosphodiesterase-4B (PDE4B), and pericentrin (Bradshaw and Porteous, 2011). DISC1 acts as a hub protein, interacting with many proteins (Camargo *et al*, 2007) and linking together divergent signaling pathways, such as dopamine (DA), PDE4B/cAMP, Akt/mTOR, and GSK-3/ β -catenin (Bradshaw and Porteous, 2011), all of which are involved in the neurobiology of mood disorders (Zhang, 2009; Beaulieu, 2011).

Several DISC1 mouse models have been created (Papaleo *et al*, 2012) that all show behavioral phenotypes related to human psychiatric disorders, including hyperactivity, deficient sensorimotor gating, poor working memory, reduced sociability, and behavioral despair. DISC1-Q31L mutant mice have a point mutation in the second exon (A127T) that leads to a glutamine to leucine substitution at amino acid 31 in the DISC1 protein (Q31L). The DISC1-Q31L mice are unique among other DISC1 genetic mouse models in showing a differential response to ADs *vs* antipsychotics in correcting abnormal behavior (Clapcote *et al*, 2007). The Q31L point mutation is located within the binding site for both PDE4B and GSK-3 based on peptide mapping (Mao *et al*, 2009; Murdoch *et al*, 2007). Biochemical analysis revealed that the DISC1-Q31L mutation leads to decreased PDE4B activity (Clapcote *et al*, 2007), reduced interaction with GSK-3 β and almost no binding to GSK-3 α . As a result, auto-phosphorylation of GSK-3 at Tyr279/216 sites is increased and DISC1-Q31L mutant mice have increased sensitivity to TDZD-8, a GSK-3 blocker (Lipina *et al*, 2011). Given the roles of PDE4B (Zhang, 2009) and GSK-3 (Jope and Roh, 2006; Beaulieu, 2011) in such mood disorders as bipolar disorder and depression, we sought to further investigate pathophysiological correlates of depression in the DISC1-Q31L mouse model.

The monoamine neurotransmitters (DA, serotonin, and noradrenaline) have been implicated in depression (Charney, 1998), so we measured their levels in several brain regions implicated in depression in DISC1-Q31L and wild-type (WT) mice. Given that consistent reduction of DA, serotonin and norepinephrine (NE) were found in the nucleus accumbens (NAC), a key brain area of reward (Shirayama and Chaki, 2006) and that anhedonia, the inability to experience pleasure, has been proposed as translational endophenotype of depression (Cryan and Holmes, 2005), we also assessed reward-related behavior in DISC1-Q31L mutants. Social behavior involves reward processes in different contexts, such as monogamous pair-bonding, aggression, maternal-infant attachment, or social play among juveniles (Panksepp and Lahvis, 2007). As mice prefer familiar conspecifics and form strong social attachments with familiar partners (Krames and Shaw, 1973), we hypothesized that pair-bonding among familiar cagemates would be preferable and rewarding in adult mice. Therefore, we assessed social-conditioned place preference (SCPP) in

adult DISC1-Q31L and WT mice, and the efficacy of distinct ADs (bupropion, fluoxetine, and desipramine) to correct diminished social behavior. Finally, we sought to correlate ADs effects with molecular and cellular changes in the NAC. We found that all studied ADs were able to improve social motivation and behavioral despair in DISC1-Q31L mutants, but only the DA drug (bupropion) completely restored deficient social discrimination, facilitation, and reward. In parallel, we found reduced levels of β -arrestin-1,2, cAMP-response-element-binding protein (CREB), and spine density in the NAC of DISC1-Q31L mutant mice. Bupropion, but not fluoxetine or desipramine, induced the greatest effect on CREB expression in DISC1-Q31L mutants, whereas all three ADs corrected the reduced levels of β -arrestin-1,2 and modestly ameliorated deficient spine density in the NAC. Our findings support for DISC1 as a genetic risk for depression and demonstrate the usefulness of DISC1-Q31L mutant mice as an animal model of depression that may help to develop new treatments.

MATERIALS AND METHODS

Animals

The DISC1-Q31L mutant line was backcrossed for 8–14 generations to C57BL/6J mice obtained from The Jackson Laboratory (Bar Harbor, ME). DISC1-Q31L heterozygous mice were intercrossed to generate homozygous, heterozygous, and WT animals. All experiments were performed with experimentally naïve 10–16-week-old male DISC1-Q31L homozygous mice (DISC1-Q31L) and their WT littermates. Groups of three to five same-sex littermates were housed in filtered polycarbonate cages under controlled temperature ($21 \pm 1^\circ\text{C}$), lighting (lights on: 7 am–7 pm) and humidity (50–60%). The animals were given sterile food (Purina mouse chow) and water *ad libitum*. All animal procedures were approved by the Animal Care and Use Committee of the Toronto Centre of Phenogenomics and were conducted in accordance with the requirements of the Province of Ontario Animals for Research Act 1971 and the Canadian Council on Animal Care.

Analysis of Brain Monoamines

Samples were obtained for each brain region, and tissue pellets were retained for later determination of protein content. Levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA); serotonin (5-HT) and NE were quantified using high-performance liquid chromatography with electrochemical detection. Analyses were performed on a system consisting of a Thermo Separation Products (TSP) P4000 pump (TSP, Piscataway, NJ), a TSP AS3000 autosampler with cooling unit, an ESA Coulochem II electrochemical detector (ESA 50II Analytical Cell and 5020 Guard Cell; ESA Biosciences, Chelmsford, MA) and a Spectra Physics 4290 Integrator (Spectra Physics, Irvine, CA) connected to a computer running TSP WOW chromatography software. The mobile phase, an aqueous mixture of 0.098 M glacial acetic acid, 0.09 M sodium acetate (pH 3.7), 0.118 mM ethylenediaminetetraacetic acid, 7% methanol, and 0.8 mM octane sulfonate was delivered at a flow rate of 0.8 ml/min. Separation of the 100 ml samples

was performed on an ACE 250 × 4.6 mm column with Ace C18, 5 mm stationary phase. Peak heights recorded at E2 were detected using electrode potentials as follows (Guard cell +450 mV; analytical cell E1 +100 mV; E2—400 mV). Quantification of monoamines was performed on 0.1 N perchloric acid extracts in a procedure involving two 30 min runs per sample. An appropriately diluted sample was run followed by a second run consisting of one-half sample and one-half standard cocktail (pure monoamines in concentrations of 10 pg/ml). Monoamine levels in pg/mg tissue wet weight were then calculated.

Behavioral Tests

SCPP was conducted as described previously (Panksepp and Lahvis, 2007) with adjustments to allow for testing of adult mice. DISC1-Q31L mice and WT littermates were weaned at post-natal day 21 (PD 21), separated by genotype and left until 10–12 weeks of age. On the day prior to social conditioning, mice were socially isolated from 14:00–16:00 h in individual clean cages containing corncob bedding and nesting material. Social conditioning took place over the next 3 days, which included two conditioning sessions per day (conditioning sessions occurred at 10:00–12:00 h and 16:00–18:00 h each day). During a conditioning session, mice of each genotype were either reunited or socially isolated for 20 min in one of two distinct environments (washcloth was used as an isolation-associated surface and an incontinence (absorbent) pad oriented by soft surface as material associated with social interactions) situated within each peripheral compartment of a three-chambered box (51 cm long × 25.5 cm wide × 23 cm tall). During the social conditioning sessions, each pair of mice was kept the same through the experiment, ie, each mouse was reunited with the same, familiar cagemate. These manipulations allowed us to assess the reward value of pair-bonding with a familiar partner, not just social interactions in general. Unconditioned mice from the control groups of both genotypes were socially isolated during conditioning sessions. To ensure that mice in the control groups received an equivalent amount of experience with the unconditioned stimulus (ie, social interaction), they were reunited with their respective social group once per day for 20 min in a novel, standard mouse cage (38 cm long × 20 cm wide × 15.5 cm tall) that was lined with corncob bedding and situated within a procedure room distinct from where the conditioning sessions were conducted. All variables associated with the conditioning procedure were randomized and counter-balanced across the unconditioned and conditioned groups of mice. *Habituation*: On the final day of conditioning, mice were allowed to freely explore the entire three-compartment testing arena without dividing walls for a 15 min with no conditioning cues present. On the test day, an individual mouse was placed in the central compartment of the testing arena and its behavior throughout the arena was videotaped and scored for 20 min (Observer 5.0, Noldus Information Technology, The Netherlands). The time spent in each compartment (peripheral compartments contained the socially-paired and isolation-paired materials, respectively) and the number of transitions made between each compartment during the testing session were analyzed. During the conditioning sessions, the time spent exploring

the compartment (sniffing, biting, going underneath the washcloth or absorbent pad, rearing, walking) was analyzed as an average of three sessions.

Social Affiliation and Novelty. The social behavior in this task was measured as previously described (Clapcote *et al*, 2007; Lipina *et al*, 2011), using a three-chambered box. The test mouse is placed in the center chamber with access to the two side chambers. These side chambers contain mesh cylinders that are either empty or have another mouse inside. At the beginning of each experimental session, the test mouse was placed in the central chamber and was allowed to freely explore for 5 min. Next, an unfamiliar mouse (male C57BL/6J, 'stranger 1') was placed inside a cylinder in one of the outer chambers. Time spent by the test mouse in each outer chamber was recorded over a 10 min period, to estimate social motivation (session 1). Another unfamiliar mouse (male C57BL/6J, 'stranger 2') was then placed inside an identical cylinder in the opposite outer chamber, and the activity of the test mouse was likewise recorded for a further 10 min, to evaluate social novelty (session 2). The amount of time spent near each cylinder and the number of entries into each chamber were scored using event-recording software (The Observer 5.0, Noldus Information Technology). The apparatus was cleaned with 70% ethanol after each session.

Social Interaction in a Neutral Arena

Each mouse was placed in unfamiliar neutral cage (30 cm long × 17 cm wide × 12 cm tall) filled with bedding under standard illumination and agonistic interactions were scored when the mouse encountered a weight- and age-matched unfamiliar C57BL/6J male mouse (the standard opponent). Standard opponents were used only once, and neutral cages were changed for each tested pair. The observation period started with the first interaction of the mice and lasted for 10 min. During this period, the following behaviors of the experimental animal were recorded: sniffing of the partner, following the opponent, self-grooming, freezing (sitting on one place >5 s with slight movements of head), walking, and digging of the bedding. Behavior was video-recorded and scored using event-recording software (The Observer 5.0, Noldus Information Technology).

Drug Treatments

Bupropion (4 mg/kg; Sigma), fluoxetine (5 mg/kg; Tocris) and desipramine (10 mg/kg; Sigma) were dissolved in saline (0.9% NaCl) on the day of administration and given in a volume of 10 ml/kg intraperitoneally twice per day (9 am and 6 pm) for seven days. The social conditioning sessions of SCPP occurred between injections on days 5–7. Antidepressant or saline-treated mice were tested in the SCPP, social affiliation/novelty and the forced swim test 20 h after the last injection. The doses of drugs were chosen based on the literature (Clapcote *et al*, 2007; Lipina and Roder, 2010) and preliminary experiments. For the biochemical and cellular experiments, brains were collected 20 h after the last injection following either cervical dislocation or intracardial perfusion, respectively.

Western Blots

Protein levels of β -arrestin-1,2, CREB and phosphorylation of CREB at Ser129/133. Immunoblotting was performed as previously described (Lipina *et al*, 2011). Tissue extracts from the NAC were prepared by homogenization in RIPA lysis buffer (Santa Cruz Biotechnology) containing Tris-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.004% sodium azide, PMSF, sodium-orthovanadate, protease inhibitor cocktail and phosphatase inhibitor cocktail (Sigma), followed by centrifugation at 10 000 r.p.m. for 10 min at 4 °C. Protein concentration was determined by Bradford assay (Protein Assay, Bio-Rad). The supernatants were stored at – 80 °C until analysis. Equal amounts of protein extracts (20–40 μ g) were boiled with Laemmli sample buffer and were separated on 10% or 15% (to distinguish β -arrestin-1 and β -arrestin-2) SDS PAGE and transferred to PVDF membrane (Life Sciences). Blots were immunostained overnight at 4 °C with the following antibodies: anti-phospho-CREB-Ser129/133 (1:1000, Invitrogen); anti-CREB (1:1000, Cell Signaling Technology); anti- β -arrestin-1,2 (1:5000, Cell Signaling Technology), and anti- β -actin (1:2000, Santa Cruz Biotechnology). Immune complexes were detected using appropriate peroxidase-conjugated secondary antibodies along with a chemiluminescent reagent (ThermoScientific).

Golgi-Cox Staining

Mice were anesthetized with xylazine/ketamine (10 ml/kg) and intracardially perfused with 0.9% saline. Brains were removed and immersed in Golgi-Cox solution in the dark for 14 days before transferring to 30% sucrose solution for 5 days. Sections of 200 μ m were sliced using a microtome (Leica VT1000S, Germany) and placed on 2% gelatinized microscope slides. The slides were stored in a humidified chamber for 3 days prior to further staining and fixation. Dendritic spine density was measured with Golgi-stained images captured at \times 100 magnification under bright-field illumination with a Nikon Eclipse E600 microscope. 75–100 neurons from 3–5 mice per treatment group were selected for spine analysis. Spines were counted only on the dendrites of medium spiny neurons in the NAC, as demarcated in the Golgi Atlas Of The Postnatal Mouse Brain (Valverde, 1998). Acquisition parameters were kept the same for all images and were blinded prior to analysis. Spine density was expressed as the number of spines per dendritic length (μ m).

Statistical Analysis

Behavioral, biochemical, and spine density data were analyzed using two-way ANOVA with repeated-measures using the appropriate between-subject and within-subject factors followed by Tukey's honestly significant difference (HSD) post-hoc tests. High-HPLC results were analyzed in each brain region by unpaired *t*-tests. Statistical analyses were performed using Statistica for Windows (Statsoft 5.5, Tulsa, OK). Data are expressed as mean \pm SD. A significance level of $p < 0.05$ was used for all analyses.

RESULTS

Post-Mortem Monoamine and Metabolite Levels

To examine whether the DISC1-Q31L genetic mutation might affect brain monoamines, we assessed levels of serotonin (5-HT), DA, NE, and their metabolites in the frontal cortex, striatum, hippocampus, and the NAC in DISC1-Q31L and WT mice (Table 1). There were consistent reductions of DA ($p < 0.05$), NE ($p < 0.05$), and 5-HT ($p < 0.05$) in the NAC of DISC1-Q31L mutants as compared with WT mice. In addition, levels of 3,4-dihydroxyphenylalanine (DOPA) ($p < 0.05$) and DOPAC ($p < 0.01$) were increased in the same brain area in DISC1-Q31L mice. HPLC analysis also detected increase of HVA in the frontal cortex ($p < 0.05$), decrease of HVA in the hippocampus ($p < 0.01$), increase of 5-hydroxyindoleacetic acid (HIAA) in the striatum ($p < 0.01$) and the hippocampus ($p < 0.001$) in DISC1-Q31L mutants compared with WT mice.

Social-Conditioned Place Preference

Interacting with conspecifics can be a powerful, natural reward that has been demonstrated in many species and in different conditions (Trezza *et al*, 2010). We assessed performance of DISC1-Q31L and WT mice in the SCPP task (Figure 1a and b). Analysis of exploratory behavior during the conditioning sessions revealed an effect of social facilitation to increase activity in WT mice but not in DISC1-Q31L mutants (Figure 1a). ANOVA with repeated measures revealed a main effect of genotype ($F_{1,22} = 164.8$; $p < 0.001$), social-paired conditioning ($F_{1,22} = 76.9$; $p < 0.001$), and their interactions ($F_{1,22} = 81.0$; $p < 0.001$). During the test session, socially-conditioned WT mice had a strong preference for the location where social interactions took place, whereas DISC1-Q31L mutants spent more time in the compartment where they were isolated during the conditioning sessions (Figure 1b). ANOVA with repeated measures found a significant effect of genotype ($F_{1,43} = 16.7$, $p < 0.001$), side of the social box ($F_{2,86} = 189.7$, $p < 0.001$), genotype \times social conditioning ($F_{1,42} = 25.9$; $p < 0.001$), genotype \times side of the social box ($F_{2,86} = 73.3$; $p < 0.001$), social conditioning \times side of the social box ($F_{2,86} = 28.0$; $p < 0.001$), and genotype \times social conditioning \times side of the social box ($F_{2,86} = 96.4$; $p < 0.001$) interactions. Control (unconditioned) mice of both genotypes without social- or isolation-conditioning spent an equal amount of time in each compartment in the SCPP task. Performance in the SCPP test was not affected by motor activity as the number of transitions between the compartments was comparable among genotypes (72.8 ± 14.7 and 62.4 ± 16.5 in the conditioned WT and DISC1-Q31L, and 75.7 ± 10.1 and 70.9 ± 15.6 in unconditioned WT and DISC1-Q31L groups, respectively).

Social Interactions in a Neutral Arena

In this task, sociability is assessed in a neutral arena as the response of the tested mouse to a weight- and age-matched unfamiliar C57BL/6J male mouse (the standard opponent). DISC1-Q31L mice did not follow the opponent as much as WT animals (Figure 1c; $F_{1,10} = 32.4$; $p < 0.001$) and passively avoided social contacts with the opponent (Figure 1d;

Table 1 Content of DA, NE, Serotonin, and their Metabolites (ng/mg of tissue) in the Frontal Cortex, Striatum, NAC, and Hippocampus of WT ($n = 7$) and DISC1-Q31L mice ($n = 7$)

	DOPA	DA	DOPAC	HVA	NE	NM	5-HT	5-HIAA
<i>Frontal cortex</i>								
WT	0.001 ± 0.001	0.049 ± 0.003	1.054 ± 0.183	0.114 ± 0.008	0.596 ± 0.029	0.102 ± 0.012	0.315 ± 0.013	0.255 ± 0.030
DISC1-Q31L	0.009 ± 0.005	0.062 ± 0.008	0.927 ± 0.045	0.144 ± 0.008 *	0.603 ± 0.026	0.108 ± 0.017	0.319 ± 0.022	0.326 ± 0.019
<i>Striatum</i>								
WT	0.005 ± 0.002	12.815 ± 0.284	2.103 ± 0.037	1.439 ± 0.043	0.159 ± 0.009	0	0.545 ± 0.016	0.410 ± 0.017
DISC1-Q31L	0.034 ± 0.028	13.453 ± 0.296	2.180 ± 0.074	1.652 ± 0.099	0.149 ± 0.006	0	0.543 ± 0.007	0.512 ± 0.013**
<i>Nucleus accumbens</i>								
WT	0.017 ± 0.007	9.939 ± 0.844	3.463 ± 0.064	1.715 ± 0.459	0.295 ± 0.026	0.043 ± 0.002	0.196 ± 0.023	0.163 ± 0.016
DISC1-Q31L	0.043 ± 0.009*	7.802 ± 0.372*	4.493 ± 0.204**	1.359 ± 0.076	0.222 ± 0.008*	0.039 ± 0.002	0.141 ± 0.009*	0.149 ± 0.012
<i>Hippocampus</i>								
WT	0.001 ± 0.001	0.039 ± 0.003	1.054 ± 0.183	0.114 ± 0.008	0.696 ± 0.029	0.102 ± 0.011	0.415 ± 0.013	0.255 ± 0.030
DISC1-Q31L	0.003 ± 0.003	0.022 ± 0.002	1.171 ± 0.369	0.046 ± 0.005**	0.718 ± 0.032	0.067 ± 0.004	0.526 ± 0.014	0.509 ± 0.019***

Abbreviations: DA, dopamine; DISC1, disrupted-in-schizophrenia-1; DOPA, 3,4-dihydroxyphenylalanine; DOPAC, 3,4-dihydroxy-phenylacetic acid, HVA, homovanillic acid; NAC, nucleus accumbens; NE, norepinephrine, NM, normetanephrine; WT, wild type; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ in comparison with WT mice.

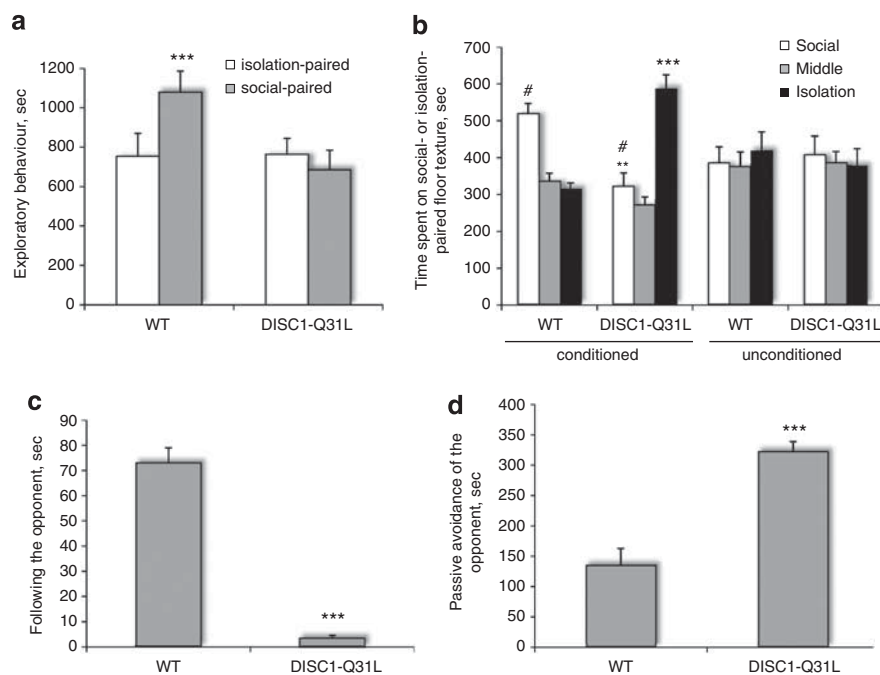


Figure 1 (a–d) Social anhedonia in DISC1-Q31L mutant mice. (a) No social facilitation was observed in DISC1-Q31L mutants ($n = 10$) during the social-conditioning sessions of the social conditioning place preference (SCPP) test. DISC1-Q31L mutants do not increase their exploration during the socially paired sessions in contrast to their WT littermates ($n = 14$ mice); *** $p < 0.001$ —in comparison with isolation-paired WT mice. (b) DISC1-Q31L mutants do not value socializing with familiar cagemates during the test session in SCPP. DISC1-Q31L mutants spent more time in the compartment where they were isolated during the conditioning sessions, whereas socially-conditioned WT mice had a strong preference for locations where social interactions took place. The number of transitions between the compartments is comparable between the genotypes. ** $p < 0.01$; *** $p < 0.001$ —in comparison with WT; # $p < 0.001$ —in comparison with isolation-paired compartment within each genotype; (c–d) DISC1-Q31L mutant mice showed social avoidance of the standard opponent assessed by Neutral Arena task. (c) Amount of time following the opponent was significantly reduced in DISC1-Q31L mutants ($n = 6$) and (d) duration of passive avoidance of the opponent was increased in DISC1-Q31L mice in comparison with WT mice ($n = 6$). *** $p < 0.001$ —in comparison with WT mice.

$F_{1,10} = 18.6$; $p < 0.001$), which is in agreement with the lack of social facilitation and deficient social reward seen in DISC1-Q31L mutants in the SCPP task.

Effects of Bupropion, Fluoxetine, and Desipramine on SCPP

Based on our finding of reduced monoamine levels in the NAC of DISC1-Q31L mutants (Table 1), we examined the ability of three different ADs that preferentially act on DA (bupropion), 5-HT (fluoxetine), or NE (desipramine) neurotransmitter systems to reverse deficient social facilitation and the reduced SCPP in DISC1-Q31L mutants

(Figure 2a and b). Analysis of ADs-treated DISC1-Q31L mice during the social-conditioning sessions found that only bupropion induced social facilitation (Figure 2a). ANOVA with repeated measures detected a main effect of the drug ($F_{3,49} = 12.8$; $p < 0.001$), genotype ($F_{1,49} = 115.6$; $p < 0.001$), social-paired conditioning ($F_{1,49}$; $p < 0.001$), and their interactions. We found that bupropion also had the most pronounced effect on DISC1-Q31L mutants in the SCPP task (Figure 2b). ANOVA found a main effect of the drug ($F_{3,49} = 3.05$; $p < 0.05$), genotype ($F_{1,49} = 4.3$, $p < 0.05$), side of the social box ($F_{2,98} = 214.8$, $p < 0.001$), and their interactions. Bupropion-treated DISC1-Q31L mice switched their preference and spent a significantly longer time on the

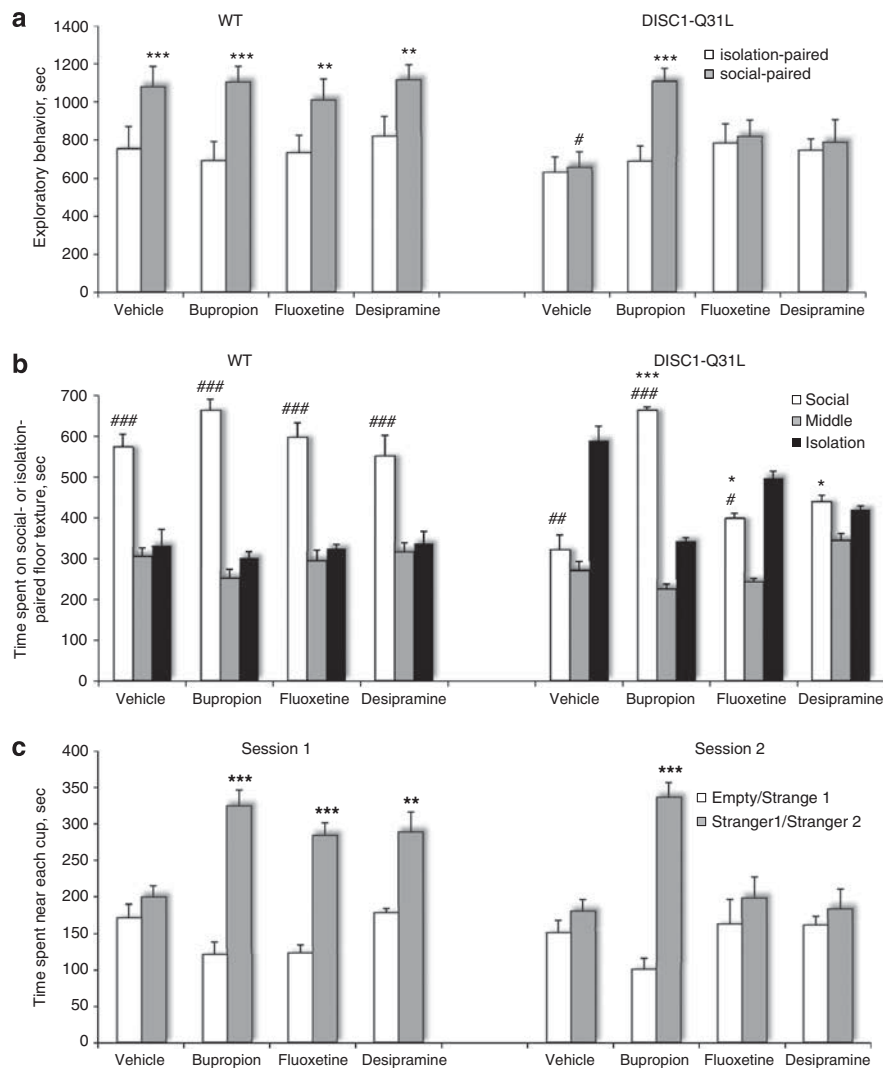


Figure 2 (a–c) Effects of bupropion, fluoxetine, and desipramine on deficient social behavior in DISC1-Q31L mice. (a) Bupropion-treated DISC1-Q31L mice significantly increased their exploratory behavior during the social-conditioning session, whereas fluoxetine- and desipramine-treated DISC1-Q31L mutants did not show social facilitation. $N = 6–8$ mice per group; # $p < 0.001$ —in comparison with social-paired WT mice; ** $p < 0.01$, *** $p < 0.001$ —in comparison with isolation-paired mice within each experimental group. (b) Only bupropion-treated DISC1-Q31L mutant mice preferred to spend time on social-paired floor texture over the isolation-associated compartment during the test session in the SCPP task, whereas fluoxetine- and desipramine-treated DISC1-Q31L mice spent equal amount of time on both compartments of the SCPP chamber. $N = 6–8$ mice per group. # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ —in comparison with isolation-paired context within each experimental group; * $p < 0.05$; *** $p < 0.001$ —in comparison with vehicle-treated DISC1-Q31L mice. (c) All three antidepressant drugs significantly improved social motivation in DISC1-Q31L mice ($N = 7–8$ mice per group) assessed in the ‘session 1’ (social affiliation), whereas only bupropion, but not fluoxetine or desipramine, improved deficient social recognition in DISC1-Q31L mutants assessed in the ‘session 2’ (social novelty). Antidepressants had no effect on motor activity of mice of both genotypes (Supplementary Table S2) and did not affect performance of WT mice (Supplementary Figure S1) ($N = 6–8$ mice per group); ** $p < 0.01$; *** $p < 0.001$ —in comparison with either time spent near the ‘empty’ cup in the ‘session 1’ or near the cup containing ‘stranger 1’ in the ‘session 2’ within each drug treatment.

social-paired floor texture ($p < 0.001$) than vehicle-treated DISC1-Q31L mice. Both fluoxetine and desipramine modestly increased the time spent in the compartment associated with social interactions and slightly reduced duration of time spent on isolation-paired floor texture, but as a result, both these drugs did not correct deficient SCPP in DISC1-Q31L mutants. There was no effect of ADs on number of transitions in each experimental group (Supplementary Table S1). None of the studied ADs affected behavior of WT mice in the SCPP task (Figure 2a-b).

Effects of Bupropion, Fluoxetine, and Desipramine on Social Affiliation/Novelty

DISC1-Q31L mutant mice consistently expressed abnormal social behavior assessed by SCPP, neutral arena, and social affiliation/novelty (Clapcote *et al*, 2007) tests. Although specific aspects of social behavior can be rewarding, it is unknown whether social proximity and/or social discrimination also impart a reward value. In this respect we compared pharmacological effects of ADs in the social affiliation/novelty task (Figure 2c). ANOVA detected a main effect of the genotype ($F_{1,51} = 17.2$; $p < 0.001$), 'stranger 1' ($F_{1,51} = 143.2$, $p < 0.001$), gene \times 'stranger 1' ($F_{1,51} = 43.8$, $p < 0.001$), drug \times 'stranger 1' ($F_{3,51} = 12.1$; $p < 0.001$), and gene \times drug \times 'stranger 1' interactions ($F_{3,51} = 33.8$; $p < 0.001$). In 'session 1' vehicle-treated DISC1-Q31L mutant mice did not prefer the chamber containing an unfamiliar conspecific mouse ('stranger 1') over the chamber containing an empty cup (Figure 2c) unlike their WT littermates (Supplementary Figure S1). Bupropion-, fluoxetine-, and desipramine-treated DISC1-Q31L mutants significantly improved their social motivation assessed in 'session 1' without any effect on number of entries (Supplementary Table S2).

ANOVA found a significant effect of genotype ($F_{1,51} = 18.3$; $p < 0.001$), 'stranger 2' ($F_{1,51} = 145.7$; $p < 0.001$), gene \times drug ($F_{3,51} = 9.3$; $p < 0.01$), gene \times 'stranger 2' ($F_{1,51} = 25.6$; $p < 0.001$), and gene \times drug \times 'stranger 2' interactions ($F_{3,51} = 8.5$; $p < 0.01$). In 'session 2', vehicle-treated DISC1-Q31L mutants did not discriminate familiar 'stranger 1' from new one 'stranger 2' (Figure 2c), whereas WT mice switched their social preference and spent more time in a chamber containing a new unfamiliar mouse ('stranger 2') (Supplementary Figure S1). Notably, only bupropion-treated DISC1-Q31L mice significantly improved their social recognition, increasing duration of time spent near 'stranger 2' ($p < 0.001$), but fluoxetine and desipramine had no effect on social recognition (Figure 2c). ADs had no effect on social behavior of WT mice assessed in both the sessions (Supplementary Figure S1) and on number of entries in both the genotypes (Supplementary Table S2).

Spine Density in the NAC of DISC1-Q31L Mutants and Effects of ADs

An emerging hypothesis suggests that the pathogenesis and treatment of depression is likely to involve altered neuronal plasticity in the hippocampus in which dendritic spines have a critical role (Calabrese *et al*, 2006). Although the NAC has not been studied as much, medium spiny neurons present in the NAC are critical for signaling by DA,

serotonin, and NE (Shirayama and Chaki, 2006). Hence, disruption of synaptic connections within these neurons could result in dysfunction of reward pathways. Therefore, we examined effects of DISC1-Q31L mutation, as well as ADs treatments on dendritic spines on medium spiny neurons within the NAC (Figure 3a-c). ANOVA found a significant main effect of genotype ($F_{1,173} = 317.81$, $p < 0.01$) and drug treatment ($F_{3,173} = 20.75$, $p < 0.01$) on spine density in the NAC. There were fewer dendritic spines in the NAC in vehicle-treated DISC1-Q31L ($p < 0.01$) compared with WT. Chronic treatments with ADs resulted in modest increases in spine density in both WT and DISC1-Q31L mice, suggesting that different types of ADs have a common effect on spine density within the NAC.

Reduced Expression of β -arrestin-1,2 and Total CREB in the NAC, but not in the Hippocampus of DISC1-Q31L Mutant Mice.

Next, we focused our efforts on β -arrestin-1,2 and the transcription factor CREB as molecular biomarkers relevant to depression (Carleton *et al*, 2005; Schreiber *et al*, 2009; Uzbek and Hughes, 2011; Ran *et al*, 2011) and DISC1 pathways (Bolger *et al*, 2003; Borg *et al*, 2010; Morris *et al*, 2003; Malagasy *et al*, 2012). Both β -arresting and CREB have been implicated in signaling pathways relevant for the pathogenesis of depression and the action of ADs (reviewed in Schreiber *et al*, 2009). The β -arresting form a complex with PDE4D (Bolger *et al*, 2003) and PKB (Akt) (Björge *et al*, (2010)), upstream of GSK-3, and these are DISC1-interacting proteins (Bradshaw and Porteous, 2011). ATF4, a molecular regulator of CREB, also directly interacts with DISC1 (Morris *et al*, 2003), which can regulate ATF4 activity (Malavasi *et al*, 2012). Hence, we aimed to probe biochemical changes induced by the DISC1-Q31L mutation in the NAC, as well as in the hippocampus, in order to detect whether biochemical alterations would be specific to the NAC.

First, we performed control experiments and probed β -arrestin-1,2 in brain lysates of β -arrestin-1 and β -arrestin-2 knockout mice as negative controls. As can be seen in Supplementary Figure S3, there was a clear absence of β -arrestin-1 or β -arrestin-2 bands probed by β -arrestin-1,2 antibodies in the corresponding knockout mouse lines, verifying the specificity of the antibodies used.

ANOVA detected a main effect of genotype on β -arrestin-1 ($F_{1,20} = 18.3$; $p < 0.05$) and on β -arrestin-2 ($F_{1,20} = 31.5$; $p < 0.01$) in the NAC (Figure 4a and b), but not in the hippocampus (Figure 4e and f). The protein levels of both β -arrestin-1 and β -arrestin-2 were significantly reduced in the NAC of DISC1-Q31L mutant mice in comparison with WT. A similar decrease in β -arrestin-1 was detected in mononuclear leukocytes of patients with depression (Schreiber *et al*, 2009), supporting our findings.

Next, we examined the phosphorylation of CREB at Ser129/133 and total expression of CREB in lysates from NAC and the hippocampus of DISC1-Q31L and WT mice. We found significantly reduced level of total CREB ($F_{1,20} = 109.6$; $p < 0.001$), but not the p-Ser129/133-CREB ($F_{1,20} = 1.3$; $p > 0.05$) in the NAC of DISC1-Q31L mutants compared with WT littermates (Figure 4a, c and d). In contrast, there were no changes in total CREB, as well

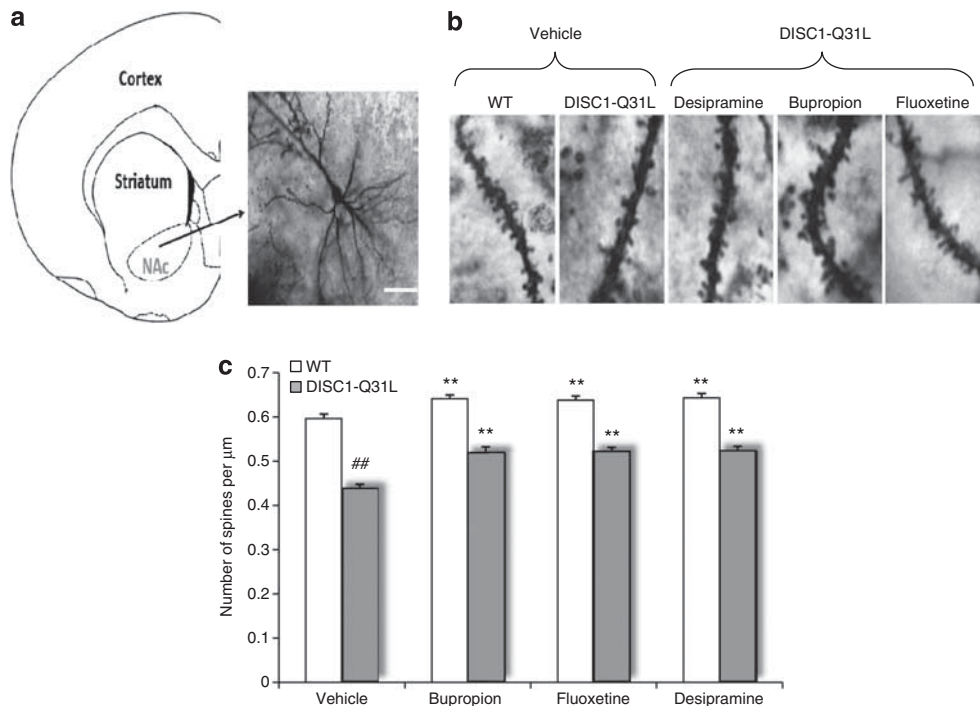


Figure 3 (a–c) The reduced spine density in the NAC of DISC1-Q31L mice was modestly meliorated by bupropion, fluoxetine, and desipramine. (a) Coronal section of mouse brain with a higher magnification image of neuron in the NAC. Golgi-stained images of spine protrusions at $\times 100$ magnification in wild-type (WT) and DISC1-Q31L mutant mice. (b) High magnification ($\times 100$) of Golgi-stained images of spine protrusions on apical dendrites. (c) Quantitative analysis of spine density in the NAC in ADs-treated DISC1-Q31L and WT mice. ^{##} $p < 0.01$ —in comparison with WT mice; ^{**} $p < 0.01$ —in comparison with vehicle-treated mice within each experimental group. $N = 75$ – 100 neurons from 3–5 mice per each treatment group.

as p-Ser129/133-CREB in the hippocampus of DISC1-Q31L mice (Figure 4e, f and h). All together, our findings highlighting specific alterations of β -arrestin-1,2 and CREB in the NAC.

Effects of Bupropion, Fluoxetine and Desipramine on β -arrestin-1,2, Total CREB and Phosphorylation of CREB at Ser 129/133 in the NAC of DISC1 Mice

Next, we sought to correlate the pharmacological effects of ADs on social behavior with molecular alterations in the NAC of DISC1 mice. ANOVA found a main effect of genotype ($F_{3,39} = 33.5$; $p < 0.001$), drug treatment, ($F_{3,39} = 48.2$; $p < 0.001$) and gene \times drug interactions ($F_{3,39} = 18.3$; $p < 0.001$) on β -arrestin-1, as well as a main effect of drug ($F_{3,39} = 51.8$; $p < 0.001$) and gene \times drug ($F_{3,39} = 20.8$; $p < 0.001$) on β -arrestin-2 protein levels (Figure 5a–c). All three ADs increased β -arrestin-1,2 levels in both genotypes. Normalization of β -arrestin-1,2 protein levels in the NAC of DISC1-Q31L mice by bupropion, fluoxetine and desipramine is consistent with clinical findings (Schreiber *et al*, 2009), where β -arrestin-1 levels in leukocytes of depressive patients have been suggested as a biomarker for treatment response.

ANOVA detected significant main effect of genotype ($F_{1,39} = 40.2$; $p < 0.001$), drug treatment ($F_{3,39} = 124.4$; $p < 0.001$), and their interactions ($F_{3,39} = 80.4$; $p < 0.001$) on the total amount of CREB in the NAC of DISC1 mice (Figure 5a and d), with no effect on pCREB at Ser129/133 ($F_{1,39} = 2.3$; $p > 0.05$ —genotype; $F_{3,39} = 0.89$; $p > 0.05$ —drug treatment) (Figure 5a and e) in WT and DISC1-Q31L mice,

consistent with previously reported data (Pascual-Brazo *et al*, 2012). Notably, bupropion elicited the most pronounced effect on CREB ($p < 0.001$) in DISC1-Q31L mutants (Figure 5a and d).

DISCUSSION

DISC1-Q31L as a Genetic Mouse Model of Depression

The neurobiology of depression is still poorly understood and new, more effective treatments are highly desirable. In the current study we examined the DISC1-Q31L genetic mouse model of depression for etiological, face, construct, and predictive validity (Cryan and Holmes, 2005). First, we identified that the NAC is a key brain area associated with social anhedonia as depression-like behavioral endophenotype in DISC1-Q31L mutants, where revealed alterations in neurochemical (reduced content of DA, serotonin, and NE), molecular (reduced levels of β -arrestin-1,2 and total CREB), and cellular (reduced spine density) function that are similar to biological endophenotypes of depression in humans (Carlezon *et al*, 2005; Schreiber *et al*, 2009; Hasler *et al*, 2004). This provides further support for construct validity of the DISC1-Q31L model. Given the importance of the NAC for reward-related behavior (Shirayama and Chaki, 2006) and depression (Treadway and Zald, 2011), the DISC1-Q31L genetic mouse offers a model to study neurobiological mechanisms of anhedonia, common to rodents and humans (Cryan and Holmes, 2005).

In the current study we found deficient social reward in DISC1-Q31L mutants that was corrected by bupropion,

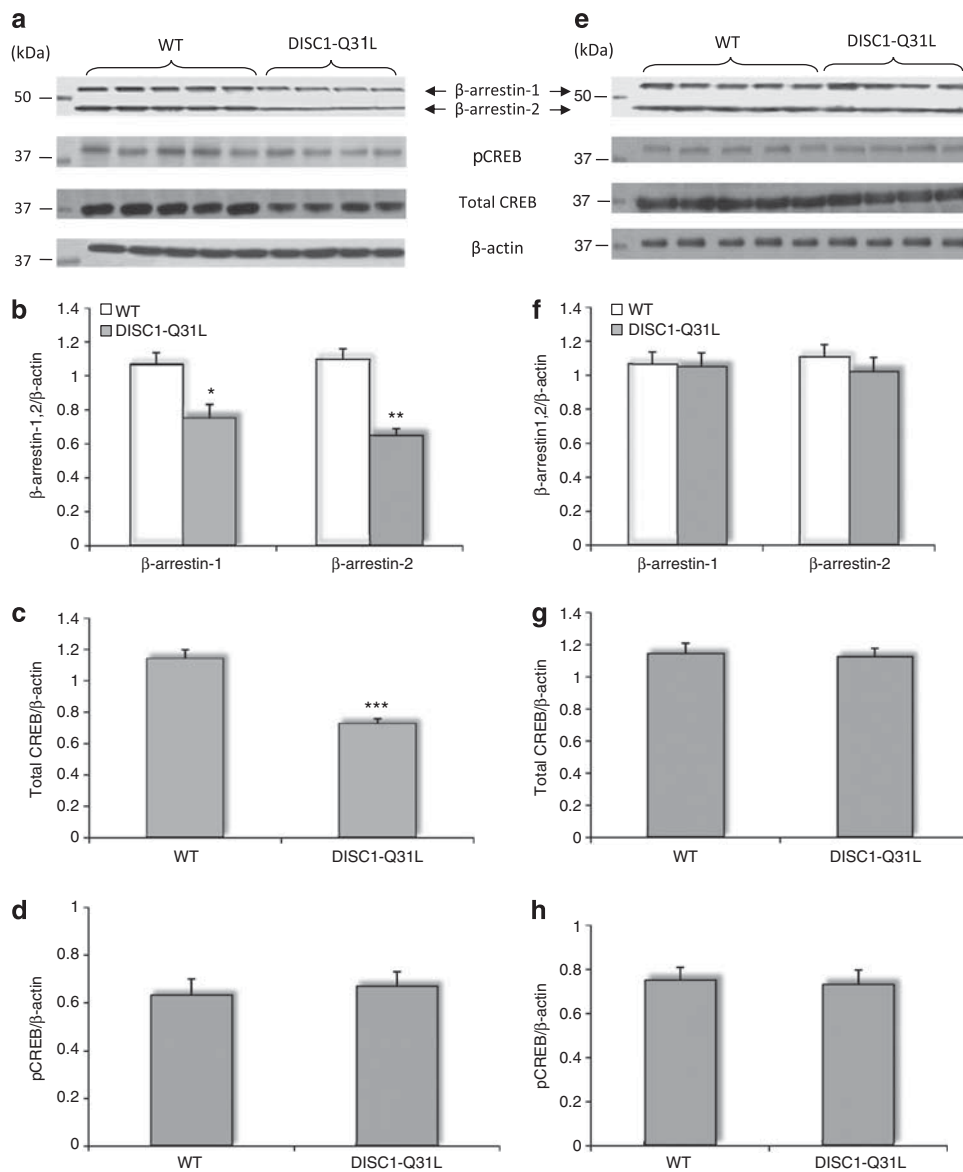


Figure 4 (a–h) The reduced protein levels of β -arrestin-1,2 and total CREB in the NAC but not in the hippocampus of DISC1-Q31L mice. (a) Representative immunoblots are shown, probed with antibodies against β -arrestin-1,2, total CREB, pCREB at Ser129/133, as well as β -actin as a loading control in the NAC of WT and DISC1-Q31L mice. Quantification of immunoblots data for β -arrestin-1, β -arrestin-2 as normalized to the total amount of β -actin (b); for pCREB at Ser129/133 as normalized to the total amount of β -actin (c), and for the total CREB as normalized to the total amount of β -actin (d). (e) Representative immunoblots are shown, probed with antibodies against β -arrestin-1,2, total CREB, pCREB at Ser129/133, as well as β -actin as a loading control in the hippocampus of WT and DISC1-Q31L mice. Quantification of immunoblots data for β -arrestin-1, β -arrestin-2 as normalized to the total amount of β -actin (f); for pCREB at Ser129/133 as normalized to the total amount of β -actin (g), and for the total CREB as normalized to the total amount of β -actin (h). $N = 8$ – 12 animals per genotype; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ —in comparison with WT.

suggesting that social anhedonia in the SSCP task might be a novel endophenotype of depression. Of course, further validations in other animal models of depression are needed to confirm this notion. Despite the ability of bupropion to correct social anhedonia and associated changes in CREB, we cannot exclude the possibility that other neurotransmitter systems and pathways are also involved in modulating depression-related behaviors in DISC1-Q31L mice. There are neurochemical changes in other brain regions (Table 1), as well as reduced spine density in the hippocampus and frontal cortex (Lee *et al*, 2011). All studied ADs we examined were able to ameliorate social motivation and

behavioral despair (Figure 2c, Supplementary Figure S2). Hence, it is important to investigate neurobiological mechanisms in other brain areas (eg, hippocampus) underlying particular behavioral endophenotypes of depression in DISC1 mutants.

Social Reward

Humans are social and the natural tendency to attend to the social world is present early in development and remains throughout life. Social rewards can cross species, as shown by chimpanzees learning a discrimination task to gain the

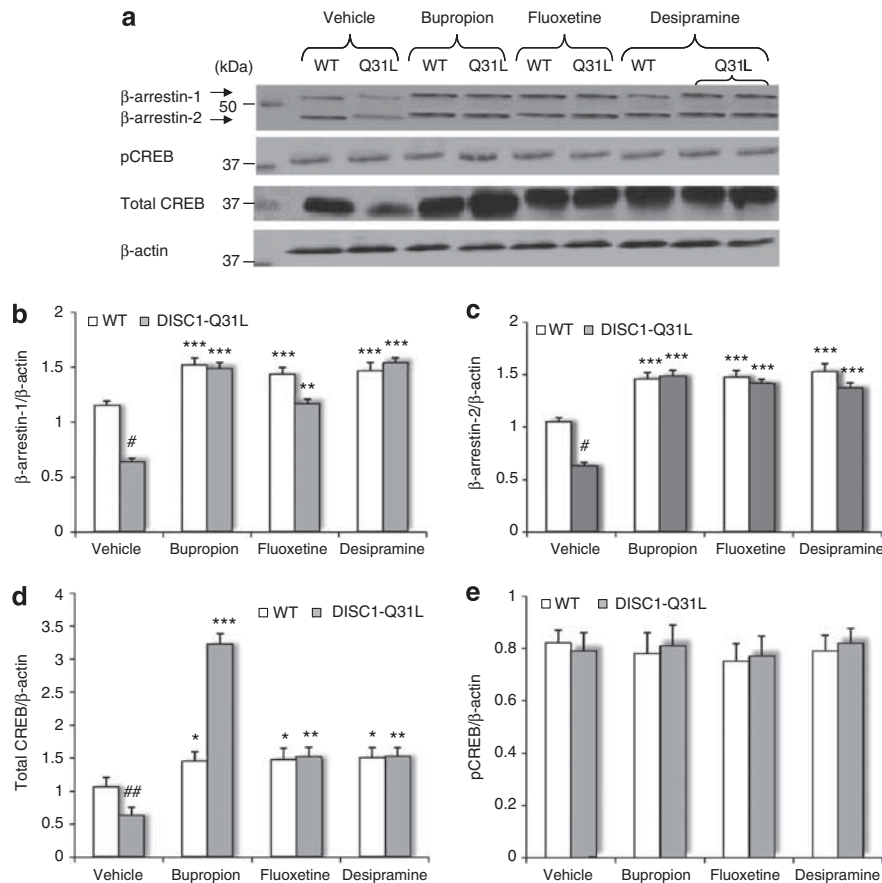


Figure 5 (a–e) Effects of bupropion, fluoxetine, and desipramine on protein amount of β -arrestin-1,2, total CREB, and phosphorylation of CREB at Ser 129/133 in the NAC of DISC1-Q31L mice. (a) Representative immunoblots are shown, probed with antibodies against β -arrestin-1,2, total CREB, pCREB at Ser 129/133, as well as β -actin as a loading control in antidepressants-treated DISC1-Q31L (Q31L) and WT mice. Quantification of immunoblots data for β -arrestin-1 and β -arrestin-2 as normalized to the total amount of β -actin (b–c); (d) for the total CREB as normalized to the total amount of β -actin and (e) for pCREB at Ser129/133 as normalized to the total amount of β -actin. $N = 6–7$ mice per each group. # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ —in comparison with vehicle-treated WT mice; * $p < 0.05$; ** $p < 0.01$; **** $p < 0.001$ —in comparison with vehicle-treated mice within each genotype.

reward of grooming the experimenter (Falk, 1958). Physical contact with another member of the same species can itself be rewarding too (Angermeier, 1960). In our study we demonstrated the reward value of interacting with a familiar partner in adult WT mice in the SCPP task. Notably, during the social-conditioning sessions, socially-paired WT mice increased their exploratory activity, demonstrating an effect of social facilitation, ie, the effect of the presence of another individual. Social facilitation influences feeding, locomotion, emotional, exploratory, and operant behavior in rodents (Wills *et al*, 1983), and occurs more frequently among familiar individuals (Krames and Shaw, 1973). In contrast, no social facilitation was observed in the conditioning sessions in DISC1-Q31L mutants and these mice did not develop SCPP. These data suggest a loss of social reward in DISC1-Q31L mutants, and that social facilitation significantly contributed to SCPP in WT mice.

ADs can influence neuronal substrates important for social behavior in depression (Novick, 2011), but are not effective in children with autism (McPheeters *et al*, 2011), another disorder associated with altered social behavior. Interestingly, there is some overlap between neural areas mediating social behavioral therapy with those affected by

ADs medications (Novick, 2011). We compared here the effects of three ADs on distinct aspects of social behavior in the DISC1-Q31L mouse model of depression. Of these ADs only bupropion corrected the deficient social reward in SCPP in DISC1-Q31L mutants, despite the fact that all three ADs rescued high immobility in the forced swim test (Supplementary Figure S2) and ameliorated deficient social motivation (Figure 2c) in DISC1-Q31L mutants. Interestingly, bupropion specifically rescued deficient social recognition (Figure 2c), which in turn induced social facilitation and led to the development of SCPP in DISC1-Q31L mice.

Such distinct efficacies of ADs on DISC1-Q31L mutants highlight the role of the mesolimbic DA system in social discrimination and social reward. Indeed, a selective blockade of DA D3 receptors enhances social novelty discrimination in rats (Watson *et al*, 2011), whereas D2 antagonists induced the opposite effect, suggesting that mutated DISC1-Q31L might affect the function of D2/D3 receptors, which are implicated in reward-seeking activity (Beaulieu and Gainetdinov, 2011) and the action of bupropion (Kitamura *et al*, 2010). Nevertheless, given that only one dose was used for each drug, perhaps higher

concentrations or/and longer periods of treatments might be needed for fluoxetine and desipramine to normalize social anhedonia in DISC1-Q31L mice.

Dopamine and Depression

Anhedonia and loss of motivation are core features of depression (Kapur and Mann, 1996) and altered DA neurotransmission in the mesolimbic system could be involved in depression (Nestler and Carlezon, 2006). Approximately 40% of patients diagnosed with major depression suffer from clinically significant anhedonia (Pelizza and Ferrari, 2009) and it is a difficult symptom to treat (Nutt *et al*, 2007; Price *et al*, 2009). However, neuroimaging, pharmacological and genetic studies strongly indicate impaired DA function in some patients with depression (Nestler and Carlezon, 2006; Dunlop and Nemeroff, 2007; Yadid and Friedman, 2008).

Indeed, analysis of brain monoamines and their metabolites in DISC1-Q31L mice revealed lower levels of DA, as well as serotonin and NE in the NAC. These findings are consistent with the idea of hypofunction of monoamine neurotransmitter systems in depression (Charney, 1998). However, decreases in DA in combination with increased levels of DOPA and the DA metabolite—DOPAC could reflect higher DA turnover in the NAC of DISC1-Q31L mutant mice. The increased amount of DA metabolite could be related to increased enzymatic activity of monoamine oxidase (MAO), which has been implicated into the pathology of depression (Lung *et al*, 2011). MAO inhibitors are used as a last line of treatment when other AD drugs are not effective (Pae *et al*, 2009). The possible upregulation of DA mesolimbic system in DISC1-Q31L mutant mice is consistent with the earlier demonstration of an increased firing of DA neurons *ex vivo* in the ventral tegmental area, which project to the NAC, in susceptible but not resilient mice chronically exposed to social defeat (Krishnan *et al*, 2007). Moreover, increased firing rates and bursting events negatively correlated with social avoidance and were reversed by fluoxetine (Cao *et al*, 2010).

Synaptic Plasticity and Depression

Dendritic spine plasticity is a critical factor of reorganization of the brain and impaired adaptive changes in spines are suggested to underlie such neuropsychiatric disorders as anxiety, depression, and drug addiction (Berton and Nestler 2006). Neuronal plasticity in the hippocampus has been extensively studied with regard to spine density and depression, with less attention paid to the NAC. However, medium spiny neurons present in the NAC are critical connections for various neurotransmitters including DA, serotonin, and NE. Hence, reduced spine density in the NAC of DISC1-Q31L mutants impairs synaptic connectivity within these neurons and could result in dysfunction of monoamine systems. Our results are consistent with previously reported reductions in dendritic spine density in the hippocampus and frontal cortex of DISC1-Q31L mutants, (Lee *et al*, 2011) and further support a role for DISC1 in modulating synaptic transmission (Bradshaw and Porteous, 2011).

However, it raises the question of the specific role of deficient spine density in the NAC for depression-related phenotypes in DISC1-Q31L mutant mice. DISC1 regulates spine formation through neurodevelopment (Bradshaw and Porteous, 2011), and likely results in a global spine deficiency in DISC1-Q31L mutants. Indeed, the inability of ADs to fully normalize the reduced spine density in adult DISC1-Q31L mice suggest that ADs might need to be given at earlier stage of development or for a longer period to fully correct spine density. The incomplete normalization of spine density by ADs treatments contrasts with complete correction of CREB, β -arrestins and deficient social behavior in DISC1 mutants. This suggests that other structural changes must underlie the behavioral effects of ADs in the NAC, or that structural changes in other brain regions such as the hippocampus should be investigated. Indeed, the hippocampus has different neurochemical and biochemical alterations than the NAC (Table 1, Figure 4a–h). Notably, the levels of DA metabolite—HVA and 5-HT metabolite—5-HIAA (both products of MAO), were significantly changed in the hippocampus of DISC1-Q31L mutants, suggesting impaired MAO function in this brain region. Given the role of MAO in depression (Lung *et al*, 2011), and that a MAO inhibitor significantly increased dendritic branching of CA3 hippocampal pyramidal neurons in primate brain (Lakshmana *et al*, 1998), it would be worth exploring the effects of ADs on spine density and biochemical cascades in the hippocampus in DISC1-Q31L mutants.

The reduced spine density in DISC1-Q31L mice would likely affect their adaptive synaptic plasticity in response to stressful events and hence, might be a critical component predisposing to the development of depression. Indeed, a range of stress procedures significantly influence the neuronal morphology of a variety of cell types, including neurons of the medial prefrontal cortex (Radley *et al*, 2006), medium spiny neurons of the NAC (Campioni *et al*, 2009; Vialou *et al*, 2010; Christoffel *et al*, 2011) or hippocampal pyramidal neurons (Magariños and McEwen, 1995; Sousa *et al*, 2000; Chen *et al*, 2010). In contrast to our findings, stress elicited different effects on spine density in distinct brain areas: induces more stubby spine structures, its functional correlate, mEPSCs (Campioni *et al*, 2009; Vialou *et al*, 2010; Christoffel *et al*, 2011), or increases AMPA/NMDA ratios (Campioni *et al*, 2009) in the NAC, but decreases spine density in the hippocampus (Magariños and McEwen, 1995; Sousa *et al*, 2000; Chen *et al*, 2010).

DISC1 Pathway and Depression

DISC1 and its network is comprised of 158 protein–protein interactions (Camargo *et al*, 2007) that regulate inter- and intra-cellular components, so point mutations such as DISC1-Q31L could affect many other proteins. Indeed, in the current study we detected effects of the DISC1-Q31L point mutation on β -arrestin-1,2, CREB, dendritic spine density, monoamine levels, and social behaviors. The β -arrestins act as scaffolds and adapters in receptor endocytosis, signal transduction via different pathways and also can translocate into the nucleus and associate with such transcription factors as p300 and CREB (Ma and Pei, 2007). β -arrestin-1,2 have been implicated in the action of AD

medications through modulating DA, serotonin and norepinephrine signaling in brain (Small *et al*, 2006; Beaulieu and Gainetdinov, 2011; Schreiber *et al*, 2009). Notably, β -arrestin-1,2 directly interacts with PDE4D (Bolger *et al*, 2003) and PKB (Akt) (Björge *et al*, 2010), upstream regulators of GSK-3 activity (Jope and Roh, 2006). GSK-3 signaling can be regulated by a β -arrestin-2 complex (Beaulieu *et al*, 2005; Beaulieu *et al*, 2007). DISC1 forms and regulates a multi-enzymatic complex with PDE4B (Millar *et al*, 2005) and GSK-3 (Mao *et al*, 2009), which act synergistically (Lipina *et al*, 2011). The reduction in β -arrestin-1,2 in DISC1-Q31L mutants is consistent with clinical findings (Avisar *et al*, 2004; Matuzany-Ruban *et al*, 2005) and suggests that DISC1 may tune the function of β -arrestin-1,2 via PDE4 and GSK-3. The β -arrestins directly regulate the function of DA receptors, DA neurotransmission and DA-related behaviors (Beaulieu and Gainetdinov, 2011), as well as modulate the signaling of 5-HT (Bohn and Schmid, 2010) and adrenergic receptors (Shenoy, 2011). Thus, the reduced β -arrestin-1,2 level specifically within the NAC, but not in the hippocampus, of DISC1-Q31L mutants could contribute to the monoaminergic imbalance in this brain region.

The transcription factor CREB, localized within the nucleus, mediates the expression of several genes (eg, *TH*, *BDNF*, *Dynorphin*, *CRF*, *GluR1*), which ultimately change the function of neuronal circuits (Carlezon *et al*, 2005). We found reduced total CREB expression in DISC1-Q31L mutants within the NAC. Given that the DISC1-Q31L mutation decreases PDE4B enzymatic activity (Clapcote *et al*, 2007), we suggest that accumulating cAMP levels in the cell could influence CREB function. The reduced level of β -arrestin-1,2 found in DISC1-Q31L mutants may also contribute to reduced CREB expression as β -arrestins can directly promote CREB transcription (Ma and Pei, 2007).

ATF4 was originally described as a transcriptional repressor, negatively regulating transcription via the CRE of the human enkephalin promoter (Karpinski *et al*, 1992) that could act as a repressor of long-term memory storage (Bartsch *et al*, 1995; Chen *et al*, 2003). A recent study (Malavasi *et al*, 2012) revealed an inhibitory effect of DISC1 on ATF4 luciferase activity, an effect that was weakened by DISC1 variants 37W and 607F, suggesting that DISC1-Q31L mutation could increase ATF activity that in turn could down-regulate CREB expression. Hence, it would be interesting to further study the functional role of the DISC1-ATF4/CREB complex in mesolimbic brain areas in regards to depression-related behaviors in the DISC1-Q31L mouse. One approach could be to manipulate by protein-protein interactions and ATF4/CREB gene expression in the NAC and compare effects of such manipulations within ventral tegmental area, as another key brain region that modulates NAC function (Krishnan *et al*, 2007). Interestingly, ATF4 over-expression in the NAC of C57BL/6J inbred mice decreased their emotional reactivity and increased depression-like behavior (Green *et al*, 2008). The down-regulation of CREB expression, induced by the DISC1-Q31L mutation is similar to the effect of various stressors used to model depression (Alfonso *et al*, 2006; Nibuya *et al*, 1996), or to the CREB-deficient mice that expressed depression-like behaviors (Zubenko and Hughes, 2011). Moreover, the decreased expression of CREB has been detected in

temporal cortex (Dowlatsahi *et al*, 1999) and in neurotrophils (Ren *et al*, 2011) of drug-free depressed patients.

Conclusions

Collectively, our findings lead us to propose DISC1 as a genetic modulator of depression at several levels of organization of the nervous system. We have shown reduced levels of monoamines, deficient dendritic spine density, accompanied by reduced levels of β -arrestin-1,2 and CREB within the NAC induced by DISC1-Q31L mutation. Thus DISC1 can help to integrate the classical monoaminergic theory of depression (Charney, 1998) and the new neuroplasticity hypothesis (Berton and Nestler, 2006). Moreover, we demonstrated here that DISC1 has an important role in social behavior and that social anhedonia could be a useful phenotype for modeling depression in rodents. In summary, the DISC1-Q31L genetic mouse model offers an opportunity to further study pathological mechanisms of depression at multiple levels of analysis, including genetic, cellular, biochemical, electrophysiological, or networks of brain pathways, which could ultimately lead to more effective treatments for depression.

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DISCLOSURE

The authors declare no conflict of interest.

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