Published in final edited form as:

Neuropharmacology. 2012 March ; 62(3): 1242–1251. doi:10.1016/j.neuropharm.2011.02.003.

Mutant DISC1 affects methamphetamine-induced sensitization and conditioned place preference: a comorbidity model

Vladimir Pogorelov¹, Jun Nomura¹, Jongho Kim⁵, Geetha Kannan^{1,7}, Chunxia Yang¹, Yu Taniguchi², Bagrat Abazyan¹, Heather Valentine^{5,6}, Irina N. Krasnova⁹, Atsushi Kamiya², Jean Lud Cadet⁹, Dean F. Wong^{2,3,5,6,8}, and Mikhail V. Pletnikov^{1,3,4,7,*}

¹ Division of Neurobiology, Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21287

² Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21287

³ Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21287

⁴ Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, MD 21287

⁵ Division of Nuclear Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287

⁶ Section of High Resolution Brain PET, Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, MD 21287

⁷ Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287

⁸ Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 21287

⁹ Molecular Neuropsychiatry Branch, NIDA, NIH, DHHS, Baltimore, 21224 MD, USA

Abstract

Genetic factors involved in neuroplasticity have been implicated in major psychiatric illnesses such as schizophrenia, depression, and substance abuse. Given its extended interactome, variants in the Disrupted-In-Schizophrenia-1 (DISC1) gene could contribute to drug addiction and psychiatric diseases. Thus, we evaluated how dominant-negative mutant DISC1 influenced the neurobehavioral and molecular effects of methamphetamine (METH). Control and mutant DISC1 mice were studied before or after treatment with non-toxic escalating dose (ED) of METH. In naïve mice, we assessed METH-induced conditioned place preference (CPP), dopamine (DA) D2 receptor density and the basal and METH-induced activity of DISC1 partners, AKT and GSK-3 β in the ventral striatum. In ED treated mice, 4 weeks after METH treatment, we evaluated fear conditioning, depression-like responses in forced swim test, and the basal and METH-induced activity of AKT and GSK-3 β in the ventral striatum. We found impairment in METH-induced CPP, decreased DA D2 receptor density and altered METH-induced phosphorylation of AKT and

*Corresponding author: Mikhail V. Pletnikov, Phone: 410-502-3760, FAX: 410-614-0013, mpletnikov@jhmi.edu.

The authors declare no conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

GSK-3 β in naïve DISC1 female mice. The ED regimen was not neurotoxic as evidenced by unaltered brain regional monoamine tissue content. Mutant DISC1 significantly delayed METH ED-produced sensitization and affected drug-induced phosphorylation of AKT and GSK-3 β in female mice. Our results suggest that perturbations in DISC1 functions in the ventral striatum may impact the molecular mechanisms of reward and sensitization, contributing to comorbidity between drug abuse and major mental diseases.

Keywords

comorbidity; schizophrenia; drug abuse; methamphetamine; DISC1; AKT; GSK-3 β ; dopamine; D2 receptors

1. Introduction

Schizophrenia, psychotic disorders, and drug abuse often show comorbidity that is an increased risk of coincident manifestation in a population affected by either clinical entity. Estimates from different studies have shown 90% prevalence of smoking, 50% of cannabis, 20–30% of stimulants in patients with major psychiatric disorders, especially schizophrenia (Barnett et al., 2007; Cantor-Graae et al., 2001; Ziedonis et al., 2003). The impact of comorbidity on society and on the course and prognosis of both, the mental and acquired disorders, is being increasingly recognized (NIDA, 2008; Shaner et al., 1995; Westermeyer, 2006).

It has been proposed that substance use comorbidity in schizophrenia is a result of self-medication in an attempt to alleviate adverse symptoms of schizophrenia or side effects of the medication. However, advances in brain imaging, neuropharmacology and human genetics have suggested that increased vulnerability to addiction in schizophrenia may be related to common pathogenic mechanisms (Chambers et al., 2001, 2010). The model of the common neurobiology has stimulated research in the neuronal systems and genetic factors shared by both disorders. The neuropathology of the striatum and cortex in schizophrenia has been speculated to affect the mechanisms of reward and/or reinforcement, leading to susceptibility to abuse drugs (Chen et al., 2003; Palomo et al., 2007; Chambers et al., 2010).

Further, recent genetic findings have identified shared genetic vulnerabilities, consistent with the hypothesis that common molecular mechanisms may operate in both diseases (Namkung et al., 2005; Volkow, 2009). In particular, genes responsible for neuronal plasticity and brain development are beginning to emerge as important risk factors (e.g., Uhl et al., 2008). In light of the neurodevelopmental hypothesis of schizophrenia and related psychiatric disorders (Keshavan and Hogarty, 1999; Rapoport and Gogtay, 2010), one would suggest that such neurodevelopmental factors as DISC1 (Millar et al., 2000), neuregulin 1 (NRG1) (Banerjee et al., 2010), members of the neurexin family (NRXN 1 and 3) (Reichelt et al., 2011), and dysbindin (Bennett, 2008; Gill et al., 2010) could participate in the pathophysiology of both substance abuse and mental illnesses.

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 **Disrupted In Schizophrenia 1** and **2** (*DISC1* and *DISC2*) (Millar et al., 2000; Millar et al., 2001). *DISC1* or the region of the *DISC1* locus have 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 (Chubb et al., 2008; Hennah and Porteous, 2009; Hennah et al., 2005; Mackie et al., 2007; Sachs et al., 2005). In addition, the altered distribution of a

nuclear isoform of DISC1 has been found in subjects with substance and alcohol abuse (Sawamura, 2005).

Recent *in vitro* and *in vivo* studies have demonstrated that disturbance of DISC1 functions may impact DA neurotransmission. The point mutation in exon 2 of the *Disc1* gene has been reported to be associated with enhanced DA neurotransmission in male mice, including the increased sensitivity to the effects of amphetamine, disrupted latent inhibition, and a 2.1-fold increase in the proportion of striatal high affinity D2 receptors (Clapcote et al, 2007; Lipina et al., 2010). Disturbances in postnatal maturation of mesocortical DA projections to the medial prefrontal cortex and resultant post-pubertal behavioral alterations have been observed in mice following prenatal transient knockdown of DISC1 in developing cortex (Minae et al, 2010). Consistently, inducible expression of mutant DISC1 in forebrain neurons has been found to produce significantly decreased content of DA in prefrontal cortex of male mice (Ayhan et al, 2010). A recent *in vitro* study has also identified a role of DISC1 in regulating primary cilia, and has shown that DA receptors exhibit subtype-selective localization to these structures, providing new insights to the basic cell biology of both DISC1 and DA receptors (Marley, 2010). Notably, DISC1 has been found to directly interact with proteins of AKT/GSK-3 β signaling (Mao et al., 2009; Kim et al, 2009) critical for DA neurotransmission and associated behaviors (Beaulieu et al., 2004). Taken together, this body of evidence has led us to propose that DISC1 may be a converging point of the molecular pathways involved in schizophrenia and drug abuse. Even if genetic evidence for DISC1 in drug addiction is still weak and only now is becoming evaluated, the multiple interaction domains of DISC1 place it at the crossroads of signaling pathways relevant to both schizophrenia and drug abuse. We believe that studying a role of DISC1 in reward mechanisms and addiction-like behaviors will greatly advance our understanding of the molecular mechanisms of comorbidity. In the present study, we used methamphetamine (METH) as an example of addictive drugs.

METH is a synthetic derivative of amphetamine but due to the addition of a methyl group it has relatively high lipid solubility. This provides for a more rapid transport of the drug across the blood–brain barrier (Barr et al., 2006), and, as a result, more profound effects on the CNS. Illicit METH use has grown increasingly prevalent over the past 10 years among abused substances in Europe, Asia, and the Americas ranking only second to marijuana worldwide (United Nations Office on Drugs and Crime, 2010). In the U.S., the number of METH treatment admissions rose from 1% in 1992 to 8% in 2004 (NIDA, 2006). The primary mechanism of METH action is neurotransmitters release through DA, serotonin and norepinephrine transporters (Kuczenski et al., 1995). The mesolimbic DA system has been demonstrated to play a key role in mediating the rewarding effects of METH (Di Chiara et al., 1993). Several signaling cascades are activated by METH, including insulin receptor substrate 1 & 2, phosphatidylinositol-3-kinase (PI3K), V-akt murine thymoma viral oncogene homolog 1 (AKT1), phospholipase C γ (PLC γ), Glycogen synthase kinase 3 beta (GSK-3 β), extracellular signal regulated kinase 1 and 2 (ERK1/2), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) (Dietz et al., 2009). DISC1 has been found to directly interact with the AKT/GSK-3 β pathways, providing rationale for elucidating the role of DISC1 in METH-produced addiction-related behaviors. To this effect, the present study used a dominant-negative mutant DISC1 mouse model.

Although expression of mutant DISC1 due to the chromosomal translocation in the Scottish pedigree has not been conclusively demonstrated or refuted (Ishizuka et al, 2006), we use mutant DISC1 as a molecular tool to affect functions of endogenous mouse DISC1. Both dominant-negative and haploinsufficiency mechanisms are thought to be able to similarly perturb DISC1-interacting proteins complexes, resulting in a loss of function of DISC1 (Chubb et al., 2008; Kamiya et al., 2005; Porteous et al., 2006). We believe that dominant-

negative mutant DISC1 mouse models can help elucidate how functional alterations in DISC1 impacts AKT/GSK3 signaling activated by METH.

Our mouse model is based on inducible expression of mutant human DISC1 in forebrain neurons (Pletnikov et al., 2008). In this model, expression of mutant DISC1 is regulated by the CAMKII promoter and can be turned off by adding doxycycline (Pletnikov et al., 2008). Expression of mutant DISC1 on a mixed background produced no gross developmental defects, however, males exhibited significantly increased spontaneous locomotor activity, enhanced aggression, and decreased social interaction, whereas females showed poorer spatial memory in the Morris water maze and depression-like responses (Pletnikov et al., 2008; Ayhan et al, 2010).

Here we report that mutant DISC1 significantly delayed METH-induced sensitization and impaired CPP in female but not male mice. The behavioral changes were associated with decreased D2 DA receptor binding and altered AKT/GSK-3 β signaling in the ventral striatum. Our results indicate that mutant DISC1 may affect the molecular mechanisms of METH-induced behavioral neuroadaptation, contributing to comorbidity between drug abuse and psychiatric disorders.

2. Methods

2.1. Animals

Our mouse model of inducible expression of mutant human DISC1 has been previously described (Pletnikov et al., 2008). We used 2–4 month-old male and female mice. Control mice were single transgenic CAMKII-tTA mice, and mutant mice were double-transgenic mice with inducible expression of mutant DISC1 in forebrain neurons. Mice of line 1001 were used in the present study (Pletnikov et al., 2008). Mice of both tTA and DISC1 1001 lines were backcrossed to the C57BL6/J for at least 9–10 generations before the current study. All animal experiments were carried out in accordance with the JHU ACUC protocol and National Institutes of Health guide for Care and Use of Laboratory animals (NIH Pub 8023).

The general protocol for all tests used in the study is depicted in Fig. 1.

2.2. Drugs

d,l-Methamphetamine hydrochloride (generously provided by the NIDA Drug Supply Program, NIDA/NIH) was dissolved in physiological saline as 10 ml/kg body weight and injected intraperitoneally (IP). d-Amphetamine sulfate (Sigma, St. Louis, MO) was used for calibration of HPLC-MS system to measure METH and amphetamine levels in the mouse brain tissue.

2.3. Sensitization Protocol

Escalating dosing (ED) of METH was applied to mice as presented in Table 1. Evidence obtained from clinical studies suggests that most human abusers initially use lower doses of METH, administered at relatively long intervals, before progressively increasing the dose, reducing the interval between successive administrations and eventually engaging in multiple daily administrations (Angrist, 1994; Gawin and Khalsa, 1996; Kramer, 1972; Simon et al., 2002). Importantly, the gradually increased levels of METH exposure do not cause the toxic effects that are associated with high acute METH doses in humans (Angrist, 1994; Gawin and Khalsa, 1996; Huber et al, 1997; Kramer, 1972; Simon et al, 2002). Thus, to closely approximate METH abuse patterns in humans, we administered METH to mice according to a non-neurotoxic escalating dose treatment. In addition, we used a two-day

break in METH injections in attempt to replicate the temporary cessation of drug intake in humans associated with the psychostimulant withdrawal syndrome (Gawin and Kleber, 1986; Srisurapanont et al, 1999). The escalating dose models similar to one we used in the present study have been successfully used by other groups (Cadet et al., 2009a,b; Danaceau JP et al., 2007; Graham et al., 2008; Johnson-Davis et al., 2004; Segal et al., 2003).

Every morning of the ED treatment, the injection was immediately followed by open field (OF) testing to monitor sensitization of locomotor activity across days of treatment. OF chambers were plastic 40×40×40 cm rectangular boxes equipped with 16×16 infrared beams (San Diego Instruments Inc., San Diego, CA, USA). Mice were gently put into a corner of the chamber and horizontal activity was scored over 30 min as beam interruptions. One week after ED treatment, mice were injected with a challenge dose of METH (1mg/kg) and tested in OF for 30 min. Another week later, separate ED-treated groups of mice received saline injections and were tested in OF to evaluate a conditioned response.

2.4. Conditioned Place Preference (CPP)

A clear plastic apparatus measuring 40×60×20 cm and divided into 3 equal-size chambers was used. On day 1, naïve mice were allowed to freely explore the apparatus for 15 min (habituation). The next day, floors with differing textures were inserted into the two side compartments, and paintings attached to the walls of the side compartments for tactile and visual discrimination. During the 15 min trial the animals were scored for the time spent in each side compartment to determine an innate preference. During days 3 through 8, mice received daily alternating injections of saline or 0.5 or 2 mg/kg of METH and were confined for 30 min in the side compartments. METH was injected in the less preferred compartment. On day 10, the animals were placed in the middle chamber and allowed to explore the entire apparatus for 15 min. The next day, the mice were injected with the training dose of METH and again allowed to explore the apparatus for 15 min. Preferences were estimated as the time difference between METH-paired and saline-paired compartments.

2.5. Contextual fear conditioning

Three weeks after METH ED, the animals were acclimated to the room for 40 min. Fear conditioning chambers (Coulbourn Instruments, Whitehall, PA) delivered one 20-s white noise tone with a scrambled 2 s 0.5 mA footshock coterminated with the tone (training day). The next day mice were placed into the chambers for 5 min and percent of immobility was captured by FreezeScan camera software (Clever Sys., Reston, VA).

2.6. Forced swim test

One week after fear conditioning test, mice were placed into glass cylinders 15×30 cm filled with room temperature water up to 20 cm high. They were videotaped for 5 min and videos analyzed by a blind observer for the duration of immobility defined as the absence of movements or the paddling with a hind leg to keep balance on the surface.

2.7. Monoamine content after ED treatments

Separate groups of mice were treated with ED dosing and sacrificed one week later to measure regional brain tissue content of monoamine and their metabolites using HPLC-ED as previously described (Ayhan et al., 2010).

2.8. Determination of brain and plasma levels of METH and amphetamine

Sample preparation involved liquid-liquid extraction with methyl tert-butyl ether of 0.5 mL aliquots of plasma or brain tissue homogenate that were spiked with the internal standards, d5-methamphetamine and d11-amphetamine. Plasma samples were diluted 1:25 (v/v) in

human plasma. Brain tissue homogenates were prepared at a concentration of 200 ng/mL in human plasma and was subsequently diluted 1:20 or 1:100 (v/v) in human plasma. Separation was achieved on Waters X-Terra C₁₈ (50 mm × 2.1 mm i.d., 3.5 μm) using isocratic elution with acetonitrile/water mobile phase (50:50, v/v) containing 0.1% formic acid at a flow rate of 0.2 mL/min. Detection was performed using an AB SCIEX 5500 triple-quadrupole mass-spectrometric using an electrospray interface operating in positive mode and monitoring the ion transitions from m/z 150.0-->91.0 (methamphetamine), m/z 136.0-->91.0 (amphetamine), m/z 155.0-->92.0 (d5-methamphetamine), and m/z 147.0-->98.0 (d11-amphetamine). The calibration range for methamphetamine and amphetamine was from 10 to 1000 ng/mL for mouse plasma samples and from 0.012 to 1.2 μg/g for mouse brain tissue samples, with an additional dilution accounting for up to 6.0 μg/g.

2.9. DA D2R [¹¹C]-raclopride quantitative autoradiography

Treatment naive four mutant and four control mice were decapitated, their brains were quickly removed and frozen in isopentane cooled with dry ice, and stored at -80°C until sectioning. Twenty μm sections were cut through the rostral part of striatum (AP+1.9–1.4, Franklin and Paxinos, 1997) and substantia nigra (SN)/Ventral Tegmental Area (VTA) (AP-2.3–3.1, Franklin and Paxinos, 1997) on a cryostat and thaw mounted onto Superfrost Plus slides (Fisher Scientific, PA, USA). The sections were preincubated for 15 min in 50mM Tris–NaCl buffer (pH 7.4) and then incubated for 60 min with 6 nM [¹¹C]-Raclopride at room temperature. Non-specific binding was determined in the presence of 6 μM cold Raclopride (Tocris Cookson Ltd., Bristol, UK) and found to represent <10% of the total binding. Incubation was terminated and unbound ligand was removed by washing first in Tris–HCl buffer (50mM, pH 7.4) with 1% BSA at room temperature for 3 min and then twice in Tris–HCl buffer (50mM, pH 7.4) at 4°C for 3 min. Following the incubation, sections were air dried for 5 min. A series of ¹¹C-standards were created following the technique of Gatley et al., 1998 and Strome et al., 2005 by pipetting 5 μL of serial dilutions onto a 25 × 75 mm strip of Instant Thin Layer Chromatography (ITLC) plate (PALL Life sciences, MI, USA; Agilent Technologies, CA, USA) (Fig. 3a). The slides and ITLC strips were exposed to the Multisensitive phosphor screen (Perkin-Elmer, MA, USA) in standard film cassettes for 2 h. The screens were scanned at 600 dpi resolution using a Cyclone storage phosphor system (Perkin-Elmer, MA, USA) and the data captured using the system's Optiquant v4.00 software. Adjacent cryostat sections were Nissl or hematoxylin-eosin stained. The pattern of autoradiographic receptor labeling was directly compared with the anatomy in the corresponding section using a plug-in co-registration algorithm for Image J software (NIH, Bethesda, MD). In this algorithm, each landmark point produces two linear constraints, so the landmark points give rise to a linear system of equations solved by the singular value decomposition. For standards, activity was measured in circular regions of interest (ROI) of 2.5 mm diameter by ImageJ and converted to pmol/cc tissue using the standard curve, with the assumption that the volume of each ROI corresponded to that of a cylinder of radius 1.25 mm and height of 20 μm.

Autoradiographic data were analyzed with both digital light unit (DLU) per mm² and binding index (BI) to the reference region $[(ROI_{\text{structure}} - ROI_{\text{reference}})/ROI_{\text{reference}}]$. Inter-hemispheric cortex and midbrain were used as a reference for ventral striatum and SN/VTA, respectively. The DLUs were converted into pmol/cc tissue using linear fitting from the standard curves with $y=0.0030x-1.5588$, $r^2=0.9$ for Substantia Nigra (SN)/Ventral Tegmental Area (VTA), respectively. The default value of 10 was multiplied to nM/L activity due to 20μm slice thickness to make up for 200 mm thickness of standards. Six by six composite images from four by four mice were selected for blind quantification for ventral striatum including OT contiguous with NAc and the anterior part of caudate-putamen and SN/VTA, respectively.

2.10. Western blotting

Naïve mice were injected with saline or 3 mg/kg METH i.p. in the home cages and left undisturbed for 90 min after which they were sacrificed by decapitation. Brains were quickly removed and striatum was dissected out within 65 sec on ice, immediately frozen in liquid nitrogen, and kept at -80°C .

In a separate experiment, 5 weeks after METH ED, mice were injected i.p. with 1 mg/kg METH or saline and placed into the OF chambers for 10 min. Immediately after, they were sacrificed by decapitation and ventral striatum was dissected as above. The samples were assayed for expression of endogenous mouse DISC1, human mutant (myc-tagged) DISC1 (Pletnikov et al., 2008), phosphorylated and total isoforms of AKT and GSK-3 β . Membranes were probed with primary antibodies to rabbit anti-mouse DISC1 antibody for endogenous DISC1 (1:500) (Pletnikov et al., 2008), rabbit phosphor and total AKT (1:1000), GSK3 β (1:2000) and β -tubulin (1:20,000) (Cell Signaling Technology). Peroxidase-conjugated sheep anti-mouse (1:2500) antibody or donkey anti-rabbit (1:2500) (GE Healthcare) antibody were used as secondary antibodies. Images were digitized with ImageJ software (NIH, Bethesda, MD). The optical density of bands for phosphorylated proteins was normalized to that of the corresponding total proteins. The relative units were used for statistical analysis.

2.11. Statistical Analyses

Data are presented as means $\pm$ standard error of means (SEM). Analysis of variance (ANOVA) was applied to behavioral and western blot data with Day or Time as within-subjects factors and Genotype, Sex, ED (escalating dose treatment), or Dose (acute treatment) as between-subjects factors. Significant interactions were followed by simple ANOVAs and significant main effects were followed by planned T-tests or Mann-Whitney non-parametric U-test. Significance was denoted as $P < 0.05$.

3. Results

3.1. Sensitization in OF

Expression of mutant DISC1 was associated with attenuated METH ED-induced sensitization of locomotor activity in mutant female mice compared to control female animals (Fig. 2a). Repeated measures ANOVA indicated the significant Day x Genotype interaction [$F(9,306)=2.2$, $P < 0.05$], with controls showing overall higher activity after METH than mutant mice [a Genotype effect: $F(1,17)=4.4$, $P < 0.05$] (Fig. 2a). No significant differences in METH sensitization were found between control and mutant male mice (Fig. A1a). Expression of sensitization, as tested one week after ED, was evident in both sexes and not significantly different between genotypes as was the conditioned activity after saline injections (data not shown).

3.2. CPP

We found that naïve mutant female mice displayed deficient CPP to 0.5 mg/kg of METH but not to 2 mg/kg of METH [the Time x Dose interaction: $F(1,29)=12.9$, $P < 0.001$] (Fig. 2b). Simple ANOVAs showed the significant Time x Genotype interaction for 0.5 mg/kg [$F(1,19)=6.7$, $P < 0.05$] but not for 2 mg/kg [$F(1,10)=2.0$, $p > 0.05$]. Paired T-tests confirmed that female control mice developed CPP at both doses while mutant female mice showed CPP at 2 mg/kg only. In contrast, no differences in CPP were found between male control and mutant mice at both doses of METH (Fig. A1b). On next day, a subset of the same mice was given the training doses of METH (0.5 or 2 mg/kg, respectively) and tested for CPP again. This time female mutant mice developed a better preference for METH-paired

compartment (Fig. A2). Thus, a deficit in the reward conditioning in the female mutant mice parallels the delay in locomotor sensitization observed at the beginning of METH ED.

Since long-term drug use impacts the brain systems involved in cognition, stress responses, and reward sensitivity (Koob and Le Moal, 2005), we tested the effects of METH ED on fear conditioning and forced swimming test, a model for depressive-like behaviors (Porsolt et al., 1977).

3.3. Fear conditioning

No significant differences in contextual fear conditioning were observed between control and mutant ED- or saline-treated female mice (Fig. A3a). In contrast, male mutant mice showed overall less contextual freezing than control male mice irrespective of treatment [an effect of Genotype: $F(1,22)=5.5$, $P<0.05$]. In addition, there was a strong trend towards ED-induced decreased freezing in control male mice [an effect of ED: $F(1,22)=3.8$, $p=0.06$] (Fig. A3a).

3.4. Forced swim test

METH ED decreased immobility in control female mice and increased immobility in mutant female mice [the Genotype $\times$ ED interaction: $F(1,26)=10.8$, $P<0.003$]. No group-related effects of METH ED were seen in male mice (Fig. A3b).

As we have found the differences in METH-induced behaviors only in female DISC1 mice, all subsequent postmortem experiments were done in female mice.

3.5. Lack of toxicity after ED treatment

As METH can be toxic when used in binge doses (Mark et al., 2004; Schroder et al., 2003; Krasnova et al., 2009), we analyzed the effects of ED treatment in our mouse model and found no significant effects of ED METH on regional brain content of monoamines and their metabolites. This suggests that ED treatment had no overt toxicity (Table A1).

3.6. Levels of METH in brain and plasma of naive female mice

In order to evaluate whether or not the observed behavioral differences between control and mutant female mice might be related to possible group differences in METH pharmacokinetics, we measured levels of METH and its metabolite, amphetamine, in the mouse brain tissue. Table 2 shows that female mutant mice had a significantly higher concentration of METH in the plasma compared to female control mice 30 min after a single injection of 1 mg/kg of METH [$t(4)=4.3$, $P<0.05$]. There were no group-related differences in levels of METH or amphetamine in the brain tissue samples.

3.7. DA D2 receptor autoradiography in naïve mice

We observed a significant decrease in DA D2 receptor ^{11}C -raclopride binding in the olfactory tubercle and a strong trend to decreased D2 receptor binding in n. accumbens of female mutant mice compared with control female mice (Fig. 3a) but not in the dorsal aspects of the rostral striatum (data not shown) or the substantia nigra/ventral tegmentum area (Fig. 3a).

3.8. DISC1 and AKT/GSK-3 β signaling in the ventral striatum

3.8.1 Endogenous DISC1—As our prior reports have not studied expression of mutant and endogenous DISC1 in the ventral striatum, we evaluated levels of endogenous DISC1 in control and mutant mice. All striatal samples from double transgenic mutant DISC1 mice

expressed mutant DISC1 (Fig. A4). Compared to the control samples, there was a significant decrease in levels of endogenous mouse DISC1 in the mutant samples (Fig. A4).

3.8.2. The effects of METH on AKT/GSK-3 β signaling in naïve mice—As impaired CPP, decreased behavioral activity to the first injections of METH ED treatment, and decreased D2 DA receptor binding were observed in naïve DISC1 mutant mice, we hypothesized that mutant mice may have constitutive alterations in the intracellular molecular pathways that interact with DISC1 and mediate the effects of DA. Thus, we evaluated levels of phosphorylated and total isoforms of AKT and its downstream target, GSK-3 β , in naïve mice 90 min after a single acute injection of METH (3 mg/kg, ip), similar to prior studies (Beaulieu, 2004). We found an appreciable decrease in levels of phosphorylated AKT and GSK-3 β in control but not mutant DISC1 mice (Fig. 4).

3.8.3. The effects of METH ED on AKT/GSK-3 β signaling—Repeated drug exposures have been found to cause long-lasting neuroadaptations that may constitute a basis for craving and relapse even after a protracted period of withdrawal (Koob, 2009; Valjent et al., 2006). We evaluated the effects of METH ED on AKT/GSK-3 β signaling activated by psychostimulants (Dietz et al., 2009).

In control chronically saline-treated mice, 1 mg/kg of METH produced a significant increase in levels of phosphorylated AKT, while no significant changes in levels of phosphoAKT were observed in control METH-sensitized females (Fig. 5a) [the ED x Dose interaction: $F(1,12)=7.65$, $P<0.05$]. There were no changes in levels of AKT phosphorylation in any group of mutant female mice (Fig 5a).

METH ED had a significant inhibitory effect on levels of phosphorylated GSK-3 β in mice [an effect of ED: $F(1,28)=8.5$, $P<0.007$]. A challenge with 1 mg/kg of METH significantly decreased levels of GSK-3 β phosphorylation in METH ED-treated control but not mutant female mice, $p<0.05$ (Fig. 5b).

We also measured the locomotor activity of mice in OF during 10 min after saline or METH challenge injections to see how METH-induced activity would correlate with the changes in phosphorylation of AKT or GSK-3 β (Fig. A5). There was a significant effect of ED, $F(1,29)=7.4$, $P<0.05$. A challenge with 1.0 mg/kg of METH produced a significantly increased ambulatory activity in METH-sensitized control mice compared to METH-sensitized mutant mice [$t(5)=-2.8$, $P<0.05$], in parallel with the significant decrease in levels of phosphoGSK-3 β in METH-sensitized control mice.

4. Discussion

Our study demonstrates that expression of mutant DISC1 was associated with impaired METH-induced CPP, delayed behavioral sensitization, decreased density of DA D2 receptors and altered AKT/GSK3 signaling in the ventral striatum in mutant DISC1 mice. The results indicate that perturbations in DISC1 functions could impact the signaling that may underlie reward and drug-induced neuroplasticity, contributing to comorbidity between substance abuse and major psychiatric disorders. Recent studies have suggested that substance abuse and schizophrenia may share genes that encode for proteins involved in neurodevelopment (Namkung et al., 2005; Uhl, 2008). We think that *DISC1* is a promising gene for comorbidity research as it has been implicated in neurodevelopment and major psychiatric diseases and interacts with key signaling molecules involved in drug-induced neuroplasticity.

Our findings clearly indicate sex-dependent effects of METH in control and mutant DISC1 mice. We found that mutant female mice exhibited delayed sensitization and impaired METH-induced CPP. It is unlikely that the abnormal sensitization and CPP were due to non-specific alterations in locomotor activity or learning in female DISC1 mice as female mutant mice displayed robust fear conditioning but did not demonstrate CPP at the METH dose of 0.5 mg/kg. This double dissociation suggests that the lack of CPP in female mutant DISC1 mice may be due to abnormal reward processes in the ventral striatum (Chiang et al., 2009). Since female DISC1 mice develop CPP at a higher METH dose (2 mg/kg), one can speculate about delayed rather than impaired sensitization in mutant mice. Repeated exposure to a number of psychoactive drugs facilitates development of CPP (Lett, 1989) and it is possible that, with an increased duration of training, mutant female mice could become sufficiently sensitized to express CPP to 0.5 mg/kg METH. The state-dependent learning, whereby a METH-paired compartment becomes novel and more attractive in the drug-free test, may be excluded as the other groups continued to show the same preferences after injection of the training dose of METH.

The present data are in line with our prior findings about sex-dependent neurobehavioral abnormalities in mutant DISC1 mice (Pletnikov et al., 2008; Ayhan et al., 2010). In the context of METH addiction, our study is consistent with the wealth of animal and human data about gender-related responses to psychostimulants, mostly attributed to the influence of ovarian hormones on DA neurotransmission in the brain (Robinson and Becker, 1986). One of the potential mechanisms whereby estrogens could influence METH-induced neuroadaptation includes interactions between Kalirin 7 and DISC1 (Hayashi-Takagi et al, 2010). As Kalirin 7 has been shown to be involved in cocaine-induced and estrogen-stimulated dendritic spine maturation (Penzes, 2008; Ma et al., 2010; Kiraly et al., 2010) and Kalirin 7-DISC1 interactions have been demonstrated to play an important role in dendritic spine formation (Hayashi-Takagi et al, 2010), one could speculate that METH-induced behavioral alterations in DISC1 female mice could in part result from abnormal DISC1-Kalirin 7 interactions. Future research will address this intriguing possibility.

Although the use of a single concentration of [^{11}C]-raclopride does not allow us to interpret the present binding results as change in receptors numbers vs. affinity, the concentration of the ligand of 6 nM is expected to lead to occupation of a high percentage of receptors sufficient for a reliable analysis of the data (de Araujo et al, 2009). We found that expression of mutant DISC1 in naïve mice were associated with a decrease in DA D2 receptor binding in the ventral striatum that play an important role in mediating the motor (Pijnenburg et al., 1976) and rewarding (Ikemoto et al., 2005; Selling et al., 2006) effects of stimulants. It is conceivable that decreased density of D2 DA receptors may have contributed to delayed sensitization and impaired CPP in mutant DISC1 mice. We think that the observed regional differences in DA D2 receptor binding (i.e., decreased in the ventral striatum and somewhat increased in the VTA/SN area) may be related to the regional activity of the CAMKII promoter that has been shown to be predominantly active in forebrain neurons (Pletnikov et al, 2008). The down-regulation of endogenous DISC1 found in striatum of mutant DISC1 mice corroborates our previous findings of the decreased expression in cortical samples (Ayhan et al, 2010), supporting our general hypothesis that mutant DISC1 may have a dominant negative effect on the endogenous counterpart.

Decreased D2 DA receptors binding in the ventral but not dorsal striatum is a feature of impulsive rats that are susceptible to escalating doses of cocaine self-administration (Dalley et al., 2007). This may seem opposite to our findings in mutant DISC1 mice that exhibited impaired METH-induced sensitization and CPP. Down-regulation of DA D2 receptors has been also observed after high repeated doses of psychostimulants (Chen et al., 1999). Since neuroadaptation to drugs of abuse and the mechanisms that predispose to self-administration

are different, one could interpret our results as a state of “adapted” DA D2 signaling due to disruption of endogenous DISC1, making mutant mice less sensitive to effects of METH. The opposite data, however, have been reported for mice with the point mutation in exon 2 of *Disc1* (Clapcote et al., 2007; Lipina et al., 2010), i.e., increased sensitivity to low doses of amphetamine and up-regulation of DA D2 receptors in the striatum of male mice. It is conceivable that the missense mutation and mutant DISC1 may have different effects on neuronal plasticity.

The mechanisms of dominant-negative effects of mutant DISC1 may involve a disruption of interaction of endogenous DISC1 with GRB2, an adaptor protein for DA D2-like receptors (Oldenhof et al., 1998). Alternatively, mutant DISC1 could affect interactions between endogenous DISC1 and a host of DA receptors interacting proteins involved in regulation of DA D2 receptor trafficking (Kabbani and Levenson, 2007). For example, an agonist-induced D2 receptor internalization may be significantly reduced if the function of arrestin-2 becomes compromised (Macey et al., 2004). Importantly, changes in endogenous DISC1 may also affect signaling pathways downstream of D2 DA receptor, including AKT and GSK-3 β (Beaulieu et al., 2004) that interact with DISC1 and may be involved in the pathophysiology of mental conditions. For example, recent studies have demonstrated the suppression of phosphorylation of AKT following knockdown of endogenous DISC1 protein in primary neuronal cultures (Hashimoto, 2006), direct interactions of DISC1 with GSK-3 β (Mao et al., 2009) or KIAA1212, an AKT binding partner (Kim et al., 2009). There have been also reports to suggest a role of AKT/GSK3 β signaling in schizophrenia, depression, psychosis and responses to lithium (Beaulieu et al., 2009; Benedetti et al., 2004; Mao et al., 2009; Karege et al., 2007; Serretti et al., 2008; Feyberg, 2010) as well as in neurodevelopment (Hur and Zhou, 2010), and NMDA-dependent synaptic plasticity (Peineau et al., 2008) that can be affected by chronic exposure to drugs of abuse (Russo et al., 2010).

We evaluated some aspects of AKT/GSK3 β signaling to begin elucidating the mechanisms of abnormal CPP and sensitization in mutant DISC1 mice. We found that acute treatment with METH decreased levels of phosphorylated AKT and GSK-3 β in naïve control mice without altering those in naïve mutant DISC1 mice at 90 min post-treatment. These results seem consistent with a previous study that has reported an amphetamine-induced decrease in phosphorylation of AKT and GSK-3 β in the mouse striatum (Beaulieu et al., 2004). One could hypothesize that alterations in DISC1 may render AKT/GSK-3 β signaling less sensitive to psychostimulants, leading to delayed sensitization and impaired CPP. After METH ED treatments, a challenge with a single injection of METH decreased GSK-3 β phosphorylation in control but not mutant mice, further suggesting alterations in GSK-3 β signaling. This may in part explain the absence of sensitization in METH ED-treated mutant mice after a protracted period of abstinence (Fig. A5). Future studies with this model will evaluate the AKT/GSK-3 pathway in greater detail.

In a broad context, the current study is similar to those where METH-induced neuroadaptation has been studied in other genetic mouse models with mutations relevant to psychiatric disorders and/or DA signaling. For example, behavioral sensitization after repeated METH treatment has been shown to be significantly reduced in *Sandy* mice, indicating that dysbindin may have a role in the development of behavioral sensitization (Nagai et al., 2010). Mice with the deletion of FEZ1 (fasciculation and elongation protein zeta 1), another DISC1 partner, have been demonstrated to exhibit enhanced responses to METH (Sakae et al., 2008), supporting the idea that dynamic changes in the balance between levels of anti-addictive and pro-addictive factors in the brain could determine susceptibility to substance dependence (Niwa et al., 2008).

In conclusion, our study indicates that perturbations in DISC1 functions in the ventral striatum may impact the molecular mechanisms of reward and drug-induced neuroadaptation, contributing to between substance abuse and major mental illnesses. This seems to be a fertile area for the future research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The study was supported by ARRA RO1 NIMH (MVP), NARSAD (MVP), The Cell Science Research Foundation Japan (JN) and NIBIB/NIDA/NIAAA Training grant for Clinician Scientists in Imaging Research (5T32EB006351-05) (JK), and by RO1 NIMH-091230 (AK).

The authors thank Drs Michelle Rudek-Renaut and Ming Zhao for the superb technical help with measurements of methamphetamine and amphetamine in the brain tissue at the Analytical Pharmacology Core of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. The core is supported by NIH grant P30 CA006973 and the Shared Instrument Grant 1S10RR026824-01. This core contribution to the current study was made possible by Grant Number UL1 RR 025005 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. The results of the core activity are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.

The authors would like to thank Drs Christopher A. Ross and Akira Sawa (Hopkins) for critically reading the original manuscript, Drs Bruno Jedynak and Mr. Luwei Zhou (Hopkins) for the development of the “plug-in” algorithm for a co-registration in ImageJ (NIH, Bethesda, MD) of autoradiography and histology images, and Drs Adjal Nahimi and Albert H. Gjedde (Aarhus University, Aarhus, Denmark) for the protocol of autoradiography.

References

- Ayhan Y, Abazyan B, Nomura J, Kim R, Ladenheim B, Krasnova IN, Sawa A, Margolis RL, Cadet JL, Mori S, Vogel MW, Ross CA, Pletnikov MV. 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. *Mol Psychiatry*. 2010
- Banerjee A, Macdonald ML, Borgmann-Winter KE, Hahn CG. Neuregulin 1-erbB4 pathway in schizophrenia: From genes to an interactome. *Brain Res Bull*. 2010; 83:132–139. [PubMed: 20433909]
- Barnett JH, Werners U, Secher SM, Hill KE, Brazil R, Masson K, Pernet DE, Kirkbride JB, Murray GK, Bullmore ET, Jones PB. Substance use in a population-based clinic sample of people with first-episode psychosis. *Br J Psychiatry*. 2007; 190:515–520. [PubMed: 17541112]
- Barr AM, Panenka WJ, MacEwan GW, Thornton AE, Lang DJ, Honer WG, Lecomte T. The need for speed: an update on methamphetamine addiction. *J Psychiatry Neurosci*. 2006; 31:301–313. [PubMed: 16951733]
- Beaulieu JM, Sotnikova TD, Yao WD, Kockeritz L, Woodgett JR, Gainetdinov RR, Caron MG. Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc Natl Acad Sci U S A*. 2004; 101:5099–5104. [PubMed: 15044694]
- Benedetti F, Serretti A, Colombo C, Lorenzi C, Tubazio V, Smeraldi E. A glycogen synthase kinase 3-beta promoter gene single nucleotide polymorphism is associated with age at onset and response to total sleep deprivation in bipolar depression. *Neurosci Lett*. 2004; 368:123–126. [PubMed: 15351432]
- Bennett AOM. Dual constraints on synapse formation and regression in schizophrenia: neuregulin, neuroligin, dysbindin, DISC1, MuSK and agrin. *Aust N Z J Psychiatry*. 2008; 42:662–677. [PubMed: 18622774]
- Cantor-Graae E, Nordstrom LG, McNeil TF. Substance abuse in schizophrenia: a review of the literature and a study of correlates in Sweden. *Schizophr Res*. 2001; 48:69–82. [PubMed: 11278155]

- Chambers RA, Krystal JH, Self DW. A neurobiological basis for substance abuse comorbidity in schizophrenia. *Biol Psychiatry*. 2001; 50:71–83. [PubMed: 11526998]
- Chambers RA, Sentir AM, Conroy SK, Truitt WA, Shekhar A. Cortical-striatal integration of cocaine history and prefrontal dysfunction in animal modeling of dual diagnosis. *Biol Psychiatry*. 2010; 67:788–792. [PubMed: 19880093]
- Chen JC, Su HJ, Huang LI, Hsieh MMC. Reductions in binding and function of D2 dopamine receptors in the rat ventral striatum during amphetamine sensitization. *Life Sci*. 1999; 64:343–354. [PubMed: 10072194]
- Chen CK, Lin SK, Sham PC, Ball D, Loh EW, Hsiao CC, Chiang YL, Ree SC, Lee CH, Murray RM. Pre-morbid characteristics and co-morbidity of methamphetamine users with and without psychosis. *Psychol Med*. 2003; 33:1407–1414. [PubMed: 14672249]
- Chiang CY, Cherng CG, Lai YT, Fan HY, Chuang JY, Kao GS, Chang WT, Yu L. Medial prefrontal cortex and nucleus accumbens core are involved in retrieval of the methamphetamine-associated memory. *Behav Brain Res*. 2009; 197:24–30. [PubMed: 18722478]
- Clapcote SJ, Lipina TV, Millar JK, Mackie S, Christie S, Ogawa F, Lerch JP, Trimble K, Uchiyama M, Sakuraba Y, Kaneda H, Shiroishi T, Houslay MD, Henkelman RM, Sled JG, Gondo Y, Porteous DJ, Roder JC. Behavioral phenotypes of Disc1 missense mutations in mice. *Neuron*. 2007 May 3; 54(3):387–402. [PubMed: 17481393]
- Chubb JE, Bradshaw NJ, Soares DC, Porteous DJ, Millar JK. The DISC locus in psychiatric illness. *Mol Psychiatry*. 2008; 13:36–64. [PubMed: 17912248]
- Dalley JW, Fryer TD, Brichard L, Robinson ESJ, Theobald DEH, Laane K, Pena Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron JC, Everitt BJ, Robbins TW. Nucleus Accumbens D2/3 Receptors Predict Trait Impulsivity and Cocaine Reinforcement. *Science*. 2007; 315:1267–1270. [PubMed: 17332411]
- de Araujo NP, Fukushima DF, Grassl C, Hipolide DC, Souza-Formigoni ML, Tufik S, Frussa-Filho R. Ethanol-induced behavioral sensitization is associated with dopamine receptor changes in the mouse olfactory tubercle. *Physiol Behav*. 2009; 96:12–17. [PubMed: 18761028]
- Di Chiara G, Acquas E, Tanda G, Cadoni C. Drugs of abuse: biochemical surrogates of specific aspects of natural reward? *Biochem Soc Symp*. 1993; 59:65–81. [PubMed: 7910742]
- Dietz DM, Dietz KC, Nestler EJ, Russo SJ. Molecular mechanisms of psychostimulant-induced structural plasticity. *Pharmacopsychiatry*. 2009; 42(Suppl 1):S69–78. [PubMed: 19434558]
- Franklin, KBJPG. The mouse brain in stereotaxic coordinates. Academic Press; San Diego: 1997.
- Gatley SJ, Lan R, Volkow ND, Pappas N, King P, Wong CT, Gifford AN, Pyatt B, Dewey SL, Makriyannis A. Imaging the brain marijuana receptor: development of a radioligand that binds to cannabinoid CB1 receptors in vivo. *J Neurochem*. 1998; 70:417–423. [PubMed: 9422389]
- Gatley SJ, Ding YS, Brady D, Gifford AN, Dewey SL, Carroll FI, Fowler JS, Volkow ND. In vitro and ex vivo autoradiographic studies of nicotinic acetylcholine receptors using [18F]fluoronochloroepibatidine in rodent and human brain. *Nucl Med Biol*. 1998; 25:449–454. [PubMed: 9720662]
- Gill M, Donohoe G, Corvin A. What have the genomics ever done for the psychoses? *Psychol Med*. 2010; 40:529–540. [PubMed: 19818200]
- Hashimoto R, Numakawa T, Ohnishi T, Kumamaru E, Yagasaki Y, Ishimoto T, Mori T, Nemoto K, Adachi N, Izumi A, Chiba S, Noguchi H, Suzuki T, Iwata N, Ozaki N, Taguchi T, Kamiya A, Kosuga A, Tatsumi M, Kamijima K, Weinberger DR, Sawa A, Kunugi H. Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. *Hum Mol Genet*. 2006; 15:3024–3033. [PubMed: 16959794]
- Hayashi-Takagi A, Takaki M, Graziane N, Seshadri S, Murdoch H, Dunlop AJ, Makino Y, Seshadri AJ, Ishizuka K, Srivastava DP, Xie Z, Baraban JM, Houslay MD, Tomoda T, Brandon NJ, Kamiya A, Yan Z, Penzes P, Sawa A. Disrupted-in-Schizophrenia 1 (DISC1) regulates spines of the glutamate synapse via Rac1. *Nat Neurosci*. 2010; 13:327–332. [PubMed: 20139976]
- Hennah W, Porteous D. The DISC1 pathway modulates expression of neurodevelopmental, synaptogenic and sensory perception genes. *PLoS One*. 2009; 4:e4906. [PubMed: 19300510]
- Hennah W, Tuulio-Henriksson A, Paunio T, Ekelund J, Varilo T, Partonen T, Cannon TD, Lonnqvist J, Peltonen L. A haplotype within the DISC1 gene is associated with visual memory functions in

- families with a high density of schizophrenia. *Mol Psychiatry*. 2005; 10:1097–1103. [PubMed: 16103888]
- Hur EM, Zhou FQ. GSK3 signalling in neural development. *Nat Rev Neurosci*. 2010; 11:539–551. [PubMed: 20648061]
- Ikemoto S, Qin M, Liu ZH. The functional divide for primary reinforcement of D-amphetamine lies between the medial and lateral ventral striatum: is the division of the accumbens core, shell, and olfactory tubercle valid? *J Neurosci*. 2005; 25:5061–5065. [PubMed: 15901788]
- Ishizuka K, Paek M, Kamiya A, Sawa A. A review of Disrupted-In-Schizophrenia-1 (DISC1): neurodevelopment, cognition, and mental conditions. *Biol Psychiatry*. 2006; 59:1189–1197. [PubMed: 16797264]
- Kabbani N, Levenson R. A proteomic approach to receptor signaling: molecular mechanisms and therapeutic implications derived from discovery of the dopamine D2 receptor signalplex. *Eur J Pharmacol*. 2007; 572:83–93. [PubMed: 17662712]
- Karege F, Perroud N, Burkhardt S, Schwald M, Ballmann E, La Harpe R, Malafosse A. Alteration in kinase activity but not in protein levels of protein kinase B and glycogen synthase kinase-3 β in ventral prefrontal cortex of depressed suicide victims. *Biol Psychiatry*. 2007; 61:240–245. [PubMed: 16876135]
- Keshavan MS, Hogarty GE. Brain maturational processes and delayed onset in schizophrenia. *Dev Psychopathol*. 1999; 11:525–543. [PubMed: 10532623]
- Kim JY, Duan X, Liu CY, Jang MH, Guo JU, Pow-anpongkul N, Kang E, Song H, Ming GL. DISC1 regulates new neuron development in the adult brain via modulation of AKT-mTOR signaling through KIAA1212. *Neuron*. 2009; 63:761–773. [PubMed: 19778506]
- Kiraly DD, Ma XM, Mazzone CM, Xin X, Mains RE, Eipper BA. Behavioral and morphological responses to cocaine require kalirin7. *Biol Psychiatry*. 2010; 68:249–255. [PubMed: 20452575]
- Koob GF. Brain stress systems in the amygdala and addiction. *Brain Res*. 2009; 1293:61–75. [PubMed: 19332030]
- Koob GF, Le Moal M. Plasticity of reward neurocircuitry and the ‘dark side’ of drug addiction. *Nat Neurosci*. 2005; 8:1442–1444. [PubMed: 16251985]
- Krasnova IN, Hodges AB, Ladenheim B, Rhoades R, Phillip CG, Cesena A, Ivanova E, Hohmann CF, Cadet JL. Methamphetamine treatment causes delayed decrease in novelty-induced locomotor activity in mice. *Neurosci Res*. 2009; 65:160–165. [PubMed: 19559060]
- Kuczenski R, Segal DS, Cho AK, Melega W. Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *J Neurosci*. 1995; 15:1308–1317. [PubMed: 7869099]
- Lett BT. Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. *Psychopharmacology (Berl)*. 1989; 98:357–362. [PubMed: 2546170]
- Lipina TV, Niwa M, Jaaro-Peled H, Fletcher PJ, Seeman P, Sawa A, Roder JC. Enhanced dopamine function in DISC1-L100P mutant mice: implications for schizophrenia. *Genes Brain Behav*. 2010; 9:777–789. [PubMed: 20618446]
- Ma XM, Huang JP, Kim EJ, Zhu Q, Kuchel GA, Mains RE, Eipper BA. Kalirin-7, an important component of excitatory synapses, is regulated by estradiol in hippocampal neurons. *Hippocampus*. 2010
- Macey TA, Gurevich VV, Neve KA. Preferential Interaction between the dopamine D2 receptor and Arrestin2 in neostriatal neurons. *Mol Pharmacol*. 2004; 66:1635–1642. [PubMed: 15361545]
- Mackie S, Millar JK, Porteous DJ. Role of DISC1 in neural development and schizophrenia. *Curr Opin Neurobiol*. 2007; 17:95–102. [PubMed: 17258902]
- Mao Y, Ge X, Frank CL, Madison JM, Koehler AN, Doud MK, Tassa C, Berry EM, Soda T, Singh KK, Biechele T, Petryshen TL, Moon RT, Haggarty SJ, Tsai LH. Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3 β /beta-catenin signaling. *Cell*. 2009; 136:1017–1031. [PubMed: 19303846]
- Mark KA, Soghomonian JJ, Yamamoto BK. High-dose methamphetamine acutely activates the striatonigral pathway to increase striatal glutamate and mediate long-term dopamine toxicity. *J Neurosci*. 2004; 24:11449–11456. [PubMed: 15601951]

- Marley A, von Zastrow M. DISC1 regulates primary cilia that display specific dopamine receptors. *PLoS One*. 2010; 5:e10902. [PubMed: 20531939]
- Millar JK, Christie S, Semple CA, Porteous DJ. Chromosomal location and genomic structure of the human translin-associated factor X gene (TRAX; TSNAX) revealed by intergenic splicing to DISC1, a gene disrupted by a translocation segregating with schizophrenia. *Genomics*. 2000; 67:69–77. [PubMed: 10945471]
- Millar JK, Christie S, Anderson S, Lawson D, Hsiao-Wei Loh D, Devon RS, Arveiler B, Muir WJ, Blackwood DH, Porteous DJ. Genomic structure and localisation within a linkage hotspot of Disrupted In Schizophrenia 1, a gene disrupted by a translocation segregating with schizophrenia. *Mol Psychiatry*. 2001; 6:173–178. [PubMed: 11317219]
- Millar JK, Pickard BS, Mackie S, James R, Christie S, Buchanan SR, Malloy MP, Chubb JE, Huston E, Baillie GS, Thomson PA, Hill EV, Brandon NJ, Rain JC, Camargo LM, Whiting PJ, Houslay MD, Blackwood DH, Muir WJ, Porteous DJ. DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science*. 2005; 310:1187–1191. [PubMed: 16293762]
- Nagai T, Kitahara Y, Shiraki A, Hikita T, Taya S, Kaibuchi K, Yamada K. Dysfunction of dopamine release in the prefrontal cortex of dysbindin deficient sandy mice: an in vivo microdialysis study. *Neurosci Lett*. 2010; 470:134–138. [PubMed: 20045719]
- NIDA. National Institute on Drug Abuse Research Report Series. 2006. p. 1-8.
- NIDA. National Institute on Drug Abuse Research Report Series. 2008. p. 1-11.
- Niwa M, Yan Y, Nabeshima T. Genes and molecules that can potentiate or attenuate psychostimulant dependence: relevance of data from animal models to human addiction. *Ann N Y Acad Sci*. 2008; 1141:76–95. [PubMed: 18991952]
- Niwa M, Kamiya A, Murai R, Kubo K, Gruber AJ, Tomita K, Lu L, Tomisato S, Jaaro-Peled H, Seshadri S, Hiyama H, Huang B, Kohda K, Noda Y, O'Donnell P, Nakajima K, Sawa A, Nabeshima T. Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. *Neuron*. 2010; 65:480–489. [PubMed: 20188653]
- Oldenhof J, Vickery R, Anafi M, Oak J, Ray A, Schoots O, Pawson T, von Zastrow M, Van Tol HH. SH3 binding domains in the dopamine D4 receptor. *Biochemistry*. 1998; 37:15726–15736. [PubMed: 9843378]
- Palomo T, Archer T, Kostrzewa RM, Beninger RJ. Comorbidity of substance abuse with other psychiatric disorders. *Neurotox Res*. 2007; 12:17–27. [PubMed: 17513197]
- Peineau S, Bradley C, Taghibiglou C, Doherty A, Bortolotto ZA, Wang YT, Collingridge GL. The role of GSK-3 in synaptic plasticity. *Br J Pharmacol*. 2008; 153(Suppl 1):S428–437. [PubMed: 18311157]
- Penzes P, Jones KA. Dendritic spine dynamics--a key role for kalirin-7. *Trends Neurosci*. 2008; 31:419–427. [PubMed: 18597863]
- Phillips TJ, Kamens HM, Wheeler JM. Behavioral genetic contributions to the study of addiction-related amphetamine effects. *Neurosci Biobehav Rev*. 2008; 32:707–759. [PubMed: 18207241]
- Pijnenburg AJ, Honig WM, Van der Heyden JA, Van Rossum JM. Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. *Eur J Pharmacol*. 1976; 35:45–58. [PubMed: 3421]
- Pletnikov MV, Ayhan Y, Nikolskaia O, Xu Y, Ovanesov MV, Huang H, Mori S, Moran TH, Ross CA. Inducible expression of mutant human DISC1 in mice is associated with brain and behavioral abnormalities reminiscent of schizophrenia. *Mol Psychiatry*. 2008; 13:173–186. 115. [PubMed: 17848917]
- Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther*. 1977; 229:327–336. [PubMed: 596982]
- Porteous DJ, Thomson P, Brandon NJ, Millar JK. The genetics and biology of DISC1--an emerging role in psychosis and cognition. *Biol Psychiatry*. 2006; 60:123–131. [PubMed: 16843095]
- Rapoport JL, Gogtay N. Childhood onset schizophrenia: support for a progressive neurodevelopmental disorder. *Int J Dev Neurosci*. 2010
- Reichelt AC, Rodgers RJ, Clapcote SJ. The role of neurexins in schizophrenia and autistic spectrum disorder. *Neuropharmacology*. 2011

- Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res.* 1986; 396:157–198. [PubMed: 3527341]
- Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ. The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci.* 2010; 33:267–276. [PubMed: 20207024]
- Sachs NA, Sawa A, Holmes SE, Ross CA, DeLisi LE, Margolis RL. A frameshift mutation in *DISC1* disrupted in Schizophrenia 1 in an American family with schizophrenia and schizoaffective disorder. *Mol Psychiatry.* 2005; 10:758–764. [PubMed: 15940305]
- Sakae N, Yamasaki N, Kitaichi K, Fukuda T, Yamada M, Yoshikawa H, Hiranita T, Tatsumi Y, Kira J, Yamamoto T, Miyakawa T, Nakayama KI. Mice lacking the schizophrenia-associated protein *FEZ1* manifest hyperactivity and enhanced responsiveness to psychostimulants. *Hum Mol Genet.* 2008; 17:3191–3203. [PubMed: 18647754]
- Sawamura N, Sawamura-Yamamoto T, Ozeki Y, Ross CA, Sawa A. A form of *DISC1* enriched in nucleus: altered subcellular distribution in orbitofrontal cortex in psychosis and substance/alcohol abuse. *Proc Natl Acad Sci U S A.* 2005; 102:1187–1192. [PubMed: 15657124]
- Schroder N, O'Dell SJ, Marshall JF. Neurotoxic methamphetamine regimen severely impairs recognition memory in rats. *Synapse.* 2003; 49:89–96. [PubMed: 12740864]
- Segal DS, Kuczenski R, O'Neil ML, Melega WP, Cho AK. Escalating dose methamphetamine pretreatment alters the behavioral and neurochemical profiles associated with exposure to a high-dose methamphetamine binge. *Neuropsychopharmacology.* 2003; 28:1730–1740. [PubMed: 12865898]
- Sellings LH, McQuade LE, Clarke PB. Characterization of dopamine-dependent rewarding and locomotor stimulant effects of intravenously-administered methylphenidate in rats. *Neuroscience.* 2006; 141:1457–1468. [PubMed: 16753267]
- Serretti A, Benedetti F, Mandelli L, Calati R, Caneva B, Lorenzi C, Fontana V, Colombo C, Smeraldi E. Association between *GSK-3β* -50T/C polymorphism and personality and psychotic symptoms in mood disorders. *Psychiatry Res.* 2008; 158:132–140. [PubMed: 17976739]
- Shaner A, Eckman TA, Roberts LJ, Wilkins JN, Tucker DE, Tsuang JW, Mintz J. Disability income, cocaine use, and repeated hospitalization among schizophrenic cocaine abusers--a government-sponsored revolving door? *N Engl J Med.* 1995; 333:777–783. [PubMed: 7643886]
- Tsuang M. Schizophrenia: genes and environment. *Biol Psychiatry.* 2000; 47:210–220. [PubMed: 10682218]
- Volkow ND. Substance use disorders in schizophrenia--clinical implications of comorbidity. *Schizophr Bull.* 2009; 35:469–472. [PubMed: 19325163]
- Volkow ND, Wang GJ, Ma Y, Fowler JS, Wong C, Ding YS, Hitzemann R, Swanson JM, Kalivas P. Activation of orbital and medial prefrontal cortex by methylphenidate in cocaine-addicted subjects but not in controls: relevance to addiction. *J Neurosci.* 2005; 25:3932–3939. [PubMed: 15829645]
- Westermeyer J. Comorbid schizophrenia and substance abuse: a review of epidemiology and course. *Am J Addict.* 2006; 15:345–355. [PubMed: 16966190]
- Ziedonis D, Williams JM, Smelson D. Serious mental illness and tobacco addiction: a model program to address this common but neglected issue. *Am J Med Sci.* 2003; 326:223–230. [PubMed: 14557739]

Figure 1. Scheme of experiments

Arrows starting from the Naïve mice box refer to separate cohorts. The doses refer to those used in the test the arrow points toward.

Figure 2. Sensitization with METH ED and conditioned place preference in naïve female mice
a. Locomotor activity over days of treatment with METH ED; * denotes $P < 0.05$ vs. mutant METH ED-treated mice; $n = 8-12$ mice per group;
b. Conditioned place preference after conditioning with 0.5 ($n = 9-13$) or 2 mg/kg ($n = 6-7$) of METH; * denotes $P < 0.05$ vs. pre-exposure time difference.

Figure 3. DA D2 receptor autoradiography

- a. [^{11}C]-Raclopride binding in brain coronal slices of naïve female mice; n=4 per genotype; OT – olfactory tubercle; NAc – nucleus accumbens, SN/VTA – substantia nigra/ventral tegmentum area * denotes $P < 0.05$ vs. control mice (Mann-Whitney U-test);
- b. Phosphoimages of representative coronal slices with the standard scale;
- c. Representative phosphoimages and Nissl-stained sections merged with the regions of interest corresponding to olfactory tubercle (OT), nucleus accumbens (NAc) and the VTA/SN area.

Figure 4. AKT/GSK3β signaling in naïve mice

a. Ratios of phosphorylated to total AKT in control (C) and mutant (M) mice (n=3–5) received either saline (Sal) or METH injections of 3 mg/kg (METH 3) [the Genotype x Dose interaction: $F(1,12)=2.0$, $P=0.16$];

b. Ratios of phosphorylated to total GSK-3β in control (C) and mutant (M) mice (n=3–5) received either saline (Sal) or METH injections of 3 mg/kg (METH 3) [the Genotype x Dose interaction: $F(1,12)=2.2$, $P=0.15$];

c. Representative images of immunoblots for phosphoAKT and total AKT in mice; 0 and 3 are doses of METH;

d. Representative images of immunoblots for phosphoGSK-3β and total GSK-3β in mice; 0 and 3 are doses of METH.

The differences did not reach the statistical significance, $F(1,12)=2.0$, $P=0.16$ - the genotype by treatment interaction for AKT; $F(1,12)=2.2$, $P=0.15$ - the genotype by treatment interaction for GSK-3β.

Figure 5. AKT/GSK-3 β signaling after METH ED treatment

- a. Ratios of phosphorylated to total AKT in control (C) and mutant (M) mice (n=3–5) received either chronic saline (Sal) or METH ED (sensitized) injections followed 5 weeks later by a single challenge injection of saline (Sal) or 1 mg/kg of METH (METH 1); * denotes $P < 0.05$ vs. chronic saline control group received a saline challenge injection in OF;
- b. Ratios of phosphorylated to total GSK-3 β in control (C) and mutant (M) mice (n=4–6) received either chronic saline (Sal) or METH ED (sensitized) injections followed 5 weeks later by a single challenge injection of saline (Sal) or 1 mg/kg of METH; * denotes $P < 0.05$ vs. sensitized control group received a saline challenge injection in OF
- c. Representative images of immunoblots for phosphoAKT and total AKT in mice; 0 and 1 are doses of METH used for challenge injections in OF;
- d. Representative images of immunoblots for phosphoGSK-3 β and total GSK-3 β in mice; 0 and 1 are doses of METH used for challenge injections in OF.

The schedule of treatments with chronic escalating doses of METH

Time is presented in the 24-h format; METH doses in mg/kg, i.p. are in bold.

Table 2

Brain tissue levels of METH and amphetamine

Drug	Genotype	Striatum (µg/g)		Plasma (ng/ml)	
		30 min	2 h	30 min	2 h
METH	Control	1.73 ±0.02	0.43 ±0.01	145.2 ±8.4	40.5 ±1.9
	Mutant	1.96 ±0.3	0.44 ±0.06	175.6 ±8.8 *	42.1 ±2.0
Amphetamine	Control	0.10 ±0.02	0.06 ±0.02	12.0 ±2.3	6.6 ±1.3
	Mutant	0.11 ±0.01	0.07 ±0.01	13.6 ±1.5	7.0 ±0.2

METH and amphetamine concentrations were measured in the striatum and plasma of female control and mutant mice (n=3 per group) at 30 min or 2 h after a single injection of 1 mg/kg of METH;

* denotes P<0.05 vs. control mice.