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DISC1 signaling in cocaine addiction: towards molecular mechanisms of co-morbidity

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Abstract

Substance abuse and other psychiatric diseases may share molecular pathology. In order to test this hypothesis, we examined the role of Disrupted In Schizophrenia 1 (DISC1), a psychiatric risk factor, in cocaine self-administration (SA). Cocaine SA significantly increased expression of DISC1 in the nucleus accumbens (NAc); while knockdown of DISC1 in NAc significantly increased cocaine SA and decreased phosphorylation of GSK-3 β at Ser9 compared to scrambled shRNA. Our study provides the first mechanistic evidence of a critical role of DISC1 in drug-induced behavioral neuroadaptations and sheds more light at the shared molecular pathology of drug abuse and other major psychiatric disorders.

Keywords

cocaine; DISC1; co-morbidity; drug abuse; psychiatric disorders

Recent studies have made significant progress in uncovering molecular mechanisms of substance abuse. While the majority of studies have concentrated on dopamine (DA)- and glutamate synaptic neurotransmission (Bonci et al., 2003; Kalivas and Volkow, 2011), there have been fewer studies of the molecular factors involved in neuroplasticity associated with drug addiction (Russo et al., 2009). As the role of neuroplasticity in mental diseases is being increasingly appreciated (Costa et al., 2001; Penzes et al., 2011), evaluating contributions of

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psychiatric genetic risk factors to drug-induced neuroadaptation not only advances our knowledge of the molecular pathology of addiction, but also facilitates studies of molecular mechanisms of co-morbidity between substance abuse and other psychiatric disorders (Volkow, 2009).

Disrupted In Schizophrenia 1 (*DISC1*) is a gene disrupted by a balanced (1:11) (q42.1; q14.3) translocation, segregating in the Scottish family with schizophrenia, depression, bipolar disorder and other minor psychiatric conditions (Millar et al., 2001; Millar et al., 2000). The increased expression of a nuclear isoform of DISC1 has been found in subjects with substance and alcohol abuse (Sawamura et al., 2005). We previously demonstrated that expression of dominant-negative mutant DISC1 (a product of the Scottish translocation) attenuated methamphetamine-induced behavioral sensitization, conditioned place preference and phosphorylation of GSK-3 β in the nucleus accumbens (NAc) (Pogorelov et al., 2012). However, non-contingent administration of cocaine and other psychostimulants does not really mimic human conditions (e.g., Ahmed, 2010). Thus, the main goal of the study is to determine the role of DISC1 in cocaine-induced addictive behaviors using a self-administration (SA) technique.

We first asked the question if cocaine SA would have any effects on expression of DISC1 in the NAc, a key brain region mediating reward to drugs of abuse (Russo et al., 2009). In order to address this question, we used naïve male Sprague Dawley rats weighed approximately 300 g at the start of testing. Rats were singly housed following surgery and for the duration of the self-administration phase of the experiment in order to protect the catheter/harness assembly. Behavioral testing took place 7 days/week during the dark phase of the 12 h light-dark cycle. Food and water was continuously available. This study was conducted in accordance with the guidelines set up by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo.

Twenty-four standard experimental test chambers (MED Associates, Inc., St. Albans, VT, USA) were used as previously described (Gancarz et al., 2015). -(–) Cocaine hydrochloride, gifted by NIDA, was dissolved in sterile 0.9% saline. Cocaine solutions (1.5 mg/ml) were made on a weekly basis. Cocaine was delivered via a syringe pump; pump duration and injection volumes were adjusted according to body weight on a daily basis in order to deliver the correct dose of drug (0.3 mg/kg/infusion). 24 hours after the cessation of the SA protocol, rats were sacrificed and protein levels of endogenous rat DISC1 were measured in brain tissue samples from NAc with standard Western blot using custom-made anti-rat DISC1 antibody as previously described (Pletnikov et al., 2007). Our data demonstrate that cocaine SA produced a significant up-regulation of protein levels of endogenous DISC1 in NAc (Fig 1). Our findings for the first time demonstrated that cocaine SA affects expression of DISC1, suggesting the mechanistic involvement of DISC1 in this addictive behavior. In order to further test the hypothesis about the causal link between DISC1 and addictive behavior (i.e., cocaine self-administration), we decided to knockdown expression of *Disc1* in the NAc using lentivirus shRNA that was described in our previous studies (Hayashi-Takagi et al., 2010; Shahani et al., 2015). Specifically, we used the following shRNA: DISC1 shRNA (5'-GGCAAACACTGTGAAGTGC-3') and scrambled control shRNA (5'-GGAGCAGACGCTGAATTAC-3'). We aimed at the shell of the NAc as this part of the NAc

was strongly implicated in the circuit mechanisms of cocaine SA (Russo et al., 2009). Rats were randomly assigned to receive bilateral injections of lentivirus expressing ($n = 9$) or scrambled shRNA ($n = 10$) aimed at the shell of NAc. Injectors were set at a 10° angle (measurements from bregma taken from surface of skull: AP: +1.7, ML: +2.45, DV: -6.7) (Robison et al., 2013) and the lentivirus was manually infused at a rate of $0.2\mu\text{L}/\text{min}$ for a total of $2.0\mu\text{L}/\text{hemisphere}$. Injectors were left in place for an additional 10 min to allow for diffusion. Rats were allowed 25 days recovery prior to any testing to allow for maximal lentivirus expression. Eighteen days following lentivirus overexpression, rats were implanted with chronic indwelling jugular catheters as previously described in (Gancarz et al., 2012). Rats were allowed a 7-day recovery period following jugular catheter surgery. The catheters were flushed daily with 0.2 ml solution of enrofloxacin (4 mg/ml) mixed in a heparinized saline solution (50 IU/ml in 0.9% sterile saline) during recovery to preserve catheter patency. At the end of behavioral testing, each animal received iv infusion of ketamine hydrochloride (0.5 mg/kg in 0.05 ml) and the behavioral response was observed to verify catheter patency. Loss of muscle tone and righting reflexes served as behavioral indicators of patency. Only rats with patent catheters were used in data analysis.

During cocaine SA rats were tested for acquisition for 0.3 mg/kg/inf doses of cocaine across 12 testing days. On day 1 of testing, responses to the active alternative resulted in iv injections of cocaine according to a Fixed Ratio 1 (FR1) schedule of reinforcement followed by a 20 s time-out period. In this schedule, the first snout poke response into the active snout poke hole resulted in delivery of 0.3 mg/kg cocaine followed by 20 s when cocaine was unavailable. Infusions of cocaine were accompanied by 5 s illumination of the stimulus light above the active snout poke hole and the house light was turned off for the duration of the time-out period (20 s). Snout poke responses to the inactive alternative were recorded but resulted in no programmed consequences. Session durations were 2 hours. FR response requirements were increased every two days of testing (i.e., Day 3, FR2; Day 5, FR4; Day 7, FR6; Day 9, FR8; Day 11, FR10). Following testing, catheters were flushed and rats were returned to the colony room. Locomotor activity was recorded by an infrared motion-sensor system (AccuScan Instruments, Columbus, OH, USA) fitted outside plastic cages ($40 \times 40 \times 30$ cm) as previously described (Gancarz et al., 2015). The VersaMax animal activity monitoring software monitors the distance the animal travelled during a 30-min test.

The primary dependent measures for self-administration included number of infusions, active and inactive responses, and total distance traveled (cm, locomotor activity). Analysis of the number of cocaine infusions self-administered using a two-way repeated measures ANOVA revealed a main effect of group (DISC1/scrambled) [$F(1,199) = 29.89$, $p < 0.0001$] but no day by group interaction. Follow-up post hoc tests (Fisher's LSD) revealed DISC1 shRNA animals self-administered significantly more infusions than scrambled animals beginning at Day 5 and on, indicating that DISC1 shRNA rats acquired cocaine self-administration more readily than scrambled counterparts (Fig 2A). Analysis of the number of active responses emitted during self-administration using a Two-way repeated measures of active responses revealed a main effect of time (day of testing) [$F(11,199) = 6.238$, $p < 0.0001$] and group (DISC1/scrambled) [$F(1,199) = 34.15$, $p < 0.0001$], and no day by group interaction. Follow-up post hoc tests (Fisher's LSD) revealed DISC1 shRNA animals emitted significantly more active responses than scrambled rats at Days 9 – 12 of testing,

indicating that DISC1 shRNA rats acquired cocaine self-administration more readily than scrambled counterparts (Fig 2B). Analysis of the number of inactive responses emitted during self-administration using a two-way repeated measures ANOVA of inactive responses revealed no significant effects, suggesting that DISC1 shRNA and scrambled animals did not differ on responding to the inactive alternative (Fig 3A). In order to determine if the change in drug-related responding was due to changes in general arousal, locomotor activity was recorded for 30 minutes. An unpaired t-test revealed no significant differences in the total distance traveled (cm) in 30 min in DISC1 shRNA or scrambled rats [$t(17) = 0.8758$, $p > 0.05$] (Fig 3B). After completion of the behavioral tests, the animal were sacrificed, their brains were quickly removed and frozen on dry ice. Tissue punches were prepared from the shell of NAc for subsequent Western blotting evaluation of levels of GSK-3 β phosphorylation at Ser9, phosphorylation of which inhibits the kinase activity (Bhat, 2000). The following antibodies were used: for total GSK-3 β (Rb mAb, 27C10, #9315, Cell Signaling; 1:1000); for Phospho GSK-3 β Ser 9 (Rb mAb, D3A4, #9322, Cell Signaling; 1:1000); the secondary antibody was anti-Rb IgG (#7074; 1:2000). We found that compared to scrambled shRNA, DISC1shRNA significantly decreased Ser9 phosphorylation, suggesting that DISC1 knockdown led to increased GSK-3 β activity in the NAc and enhanced cocaine SA (Fig. 4).

Taken together, our study for the first time demonstrates that DISC1 may be casually involved in cocaine SA. We found that cocaine SA affects expression of DISC1 in the NAc and knockdown of expression of *Disc1* in the shell of the NAc leads to a significant increase in the measures of SA in rats and an associated decrease in inhibitory phosphorylation of GSK-3 β at Ser9.

Although the exact molecular mechanisms whereby DISC1 regulates cocaine SA remain obscure, our results suggest that DISC1 may inhibit signaling pathways activated by cocaine administration. This hypothesis is consistent with up-regulation of DISC1 in the NAc in rats following cocaine SA as a compensatory response to cocaine and DISC1 knockdown-induced decrease in GSK-3 β inhibitory phosphorylation at Ser9. However, one cannot rule out that cocaine might have a direct effect on DISC1 expression via transcriptional and/or post-translational regulation as it has been demonstrated for other molecular factors (Zhang et al, 2012; Kennedy et al, 2015).

One candidate pathway that could be regulated by DISC1 is AKT-GSK3 β signaling that is activated by psychostimulants (Beaulieu et al., 2009); and DISC1 has been shown to bind to GSK3 β (Mao et al., 2009). Although this binding has been demonstrated for proliferating neurons, our data indicate that DISC1 binds to GSK3 β in mature neurons of the ventral striatum (data not shown) and DISC1 knockdown decreases inhibitory phosphorylation of GSK-3 β at Ser9, suggesting increased activity of the kinase (Bhat et al., 2000) and resultant enhancement of cocaine SA. These findings are in line with a recent study about decreased GSK-3 α/β (Ser21/9) phosphorylation in both postmortem brain tissue from schizophrenia patients and in *Disc1*-L100P mutant mice, an animal model with behavioral abnormalities related to schizophrenia (Su et al., 2014).

Our present findings may seem incongruent with our prior study (Pogorelov et al., 2012) that show mutant human DISC1 that also down-regulates expression of endogenous mouse DISC1 attenuates methamphetamine-induced behavioral sensitization and conditioned place preference. Our main hypothesis is that DISC1 inhibits GSK3 β signaling activated by psychostimulants. Thus, DISC1 knockdown dis-inhibits GSK3 β activation and increases cocaine SA. Similar effects would seem to be expected from mutant DISC1 as well. We think that the opposite effects of mutant DISC1 can be at least in part explained by the fact that mutant C-terminus truncated DISC1 retains amino acid residues (195–238) that are required for direct binding to GSK-3 β (1–597) (Pletnikov et al, 2008; Mao et al, 2009). Thus, mutant DISC1 is theoretically able to bind to GSK-3 β and might further inhibit its activity in addition to endogenous DISC1. This explanation is consistent with the results that inhibition of GSK-3 β activity decreases responses to psychostimulants (Beaulieu et al., 2009; Li and Gao, 2011). The future experiments will address this hypothesis in detail.

In conclusion, the present study provides the first experimental evidence that a psychiatric risk factor, DISC1, regulates addictive behavior and helps advance future research on the molecular mechanisms of co-morbidity of drug abuse and major psychiatric disorders.

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Highlights

- Cocaine self-administration up-regulates DISC1 expression in the nucleus accumbens
- DISC1 knockdown in the nucleus accumbens increases cocaine self-administration
- DISC1 knockdown in the nucleus accumbens decreases GSK-3 β phosphorylation
- Substance abuse and other psychiatric disorders may share molecular pathology

Figure 1.

Cocaine self-administration increases DISC1 levels in NAc

A-Representative Western blotting images of expression of DISC1 in the NAc of rats after infusions of saline or cocaine;

B-Quantitative analysis of expression; data are expressed as a DISC1/actin ratio; * - $p < 0.05$ vs. saline group; two-tailed t-test; $n = 6$ in each group

Figure 2.

DISC1 knockdown increases cocaine self-administration

A-Number of cocaine infusions self-administered per 2 hours across 12 days; FR – fixed ratio;

B-Number of active responses emitted during self-administration; * denotes $p < 0.05$ vs. scrambled RNA

Figure 3.

No effects of DISC1 knockdown on inactive responses or locomotion

A-Number of inactive responses emitted during self-administration; FR – fixed ratio;

B-General locomotor activity recorded for 30 minutes

Figure 4.

DISC1 knockdown decreased phosphorylation of GSK-3β at Ser9 in NAc

A-Representative Western blotting images of total GSK-3β and phosphorylated GSK-3β at Ser9 in the NAc of rats after injections of scrambled or DISC1 shRNA;

B-Quantitative analysis of phosphorylation; data are expressed as Ser9/total GSK-3β ratio; *

- $p < 0.05$ vs. scrambled shRNA; two-tailed t-test; $n = 6$ in each group