Neuroscience Research 105 (2016) 70–74

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Rapid Communication

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

Amy Gancarz[b], Yan Jouroukhin[a], Atsushi Saito[a], Alexey Shevelkin[a], Lauren E. Mueller[b], Atsushi Kamiya[a], David M. Dietz[b,*], Mikhail V. Pletnikov[a,c,d,e,**]

[a] Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, USA
[b] The State University of New York at Buffalo, Buffalo, NY, USA
[c] Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, USA
[d] Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, USA
[e] Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, USA

ARTICLE INFO

Article history:
Received 14 July 2015
Received in revised form 3 September 2015
Accepted 7 September 2015
Available online 15 September 2015

Keywords:
Cocaine
DISC1
Co-morbidity
Drug abuse
Psychiatric disorders

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-3β 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.

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 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 **I**n **Sc**hizophrenia **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., 2000, 2001). 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-3β 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 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.

* Corresponding author.
** Corresponding author at: Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, USA. Tel.: +1 410 502 3760.
E-mail addresses: ddietz@buffalo.edu (D.M. Dietz), mpletni1@jhu.edu (M.V. Pletnikov).

http://dx.doi.org/10.1016/j.neures.2015.09.001
0168-0102/

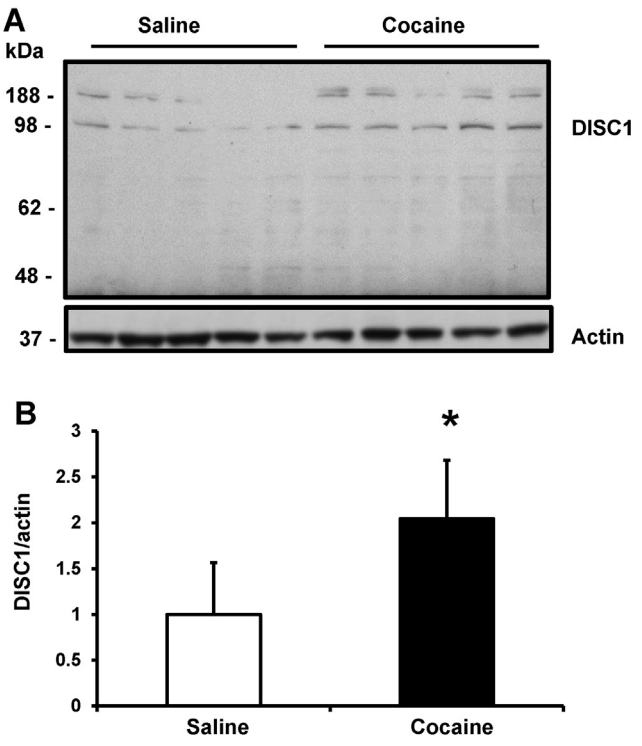

Fig. 1. Cocaine self-administration increases DISC1 levels in NAc. (A) Representative Western blotting images of expression of DISC1 in the NAc of rats after infusions of saline or cocaine; (B) quantitative analysis of expression; data are expressed as a DISC1/actin ratio; * $p < 0.05$ vs. saline group; two-tailed *t*-test; $n = 6$ in each group.

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 h 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 μL/min for a total of 2.0 μL/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 h. 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 cm × 40 cm × 30 cm) as previously described (Gancarz et al., 2015). The VersaMax animal activity monitoring software monitors the distance the animal traveled 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 min. 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 animals were sacrificed; their brains were quickly removed and frozen on dry ice. Tissue punches were prepared from the shell of NAc for subsequent