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REGULATION OF BAZ1A AND NUCLEOSOME POSITIONING IN THE NUCLEUS ACCUMBENS IN RESPONSE TO COCAINE

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Abstract—Chromatin regulation, in particular ATP-dependent chromatin remodelers, have previously been shown to be important in the regulation of reward-related behaviors in animal models of mental illnesses. Here we demonstrate that BAZ1A, an accessory subunit of the ISWI family of chromatin remodeling complexes, is downregulated in the nucleus accumbens (NAc) of mice exposed repeatedly to cocaine and of cocaine-addicted humans. Viral-mediated overexpression of BAZ1A in mouse NAc reduces cocaine reward as assessed by conditioned place preference (CPP), but increases cocaine-induced locomotor activation. Furthermore, we investigate nucleosome repositioning genome-wide by conducting chromatin immunoprecipitation (ChIP)-sequencing for total H3 in NAc of control mice and after repeated cocaine administration, and find extensive nucleosome occupancy and shift changes across the genome in response to cocaine exposure. These findings implicate BAZ1A in molecular and behavioral plasticity to cocaine and offer new insight into the pathophysiology of cocaine addiction.

Key words: cocaine, addiction, epigenetics, chromatin, chromatin remodeling.

INTRODUCTION

Chromatin regulation—in particular histone writers and erasers as well as proteins that control DNA methylation—is important for mediating the effects of repeated cocaine exposure on nucleus accumbens (NAc) gene expression and cocaine reward-related behaviors (Renthal et al., 2007; LaPlant et al., 2010; Maze et al., 2010; Covington et al., 2011; Malvaez et al., 2013; Rogge et al., 2013; Deng et al., 2014; Feng et al., 2015; Sartor et al., 2015; Wright et al., 2015; Damez-Werno et al., 2016). ATP–dependent chromatin remodelers also play an important role in regulating gene expression by altering nucleosome positioning, and have been implicated in modulating learning and memory behaviors as well as regulating reward-related behaviors in animal models of depression and drug addiction (Whitehouse et al., 2007; Clapier and Cairns, 2009; Yen et al., 2012; Narlikar et al., 2013; Vogel-Ciernia et al., 2013; Sun et al., 2015, 2016). Specifically, the persistent induction of BAZ1A, an accessory subunit of the ISWI family of chromatin remodeling proteins, has been shown to be important in mediating repeated stress-induced depressive-like behaviors through nucleosome repositioning in NAc (Sun et al., 2015). Here, we characterize the regulation of BAZ1A and nucleosome positioning in NAc after repeated cocaine exposure.

EXPERIMENTAL PROCEDURES

Animals

C57BL/6J male mice (7–8 weeks old; Jackson Laboratory, Bar Harbor, ME USA) were housed at constant temperature (23 °C) on a 12-h light/dark cycle with *ad libitum* access to food and water. All protocols were approved by Mount Sinai's IACUC unless specified otherwise.

Human postmortem brain tissue

Human NAc brain tissue was obtained from the Quebec Suicide Brain Bank (QSBB; Douglas Mental Health Institute, Verdun, Québec). All individuals were group–matched for age, pH, and postmortem intervals (PMI). Inclusion criteria for both cocaine-addicted individuals and controls were the following: the subject had to be Caucasian and of French Canadian origin, and die suddenly without prolonged agonal state. NAc was stored at −80 °C. This study was approved by the Douglas IRB and signed informed consent was obtained from next of kin.

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Abbreviations: BDNF, brain-derived neurotrophic factors; ChIP, chromatin immunoprecipitation; CPP, conditioned place preference; NAc, nucleus accumbens; PMI, postmortem intervals.

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Cocaine administration

Cocaine hydrochloride (Sigma–Aldrich, St. Louis, MO USA) was dissolved in sterile 0.9% saline (wt/vol). Daily i.p. saline or cocaine (20 mg/kg) injections were administered in the animals' home cages.

NAc RNA isolation and qRT–PCR

Total RNA was isolated from the NAc of individual mice and qRT-PCR was performed as described previously (Sun et al., 2015, 2016). Briefly, bilateral 14 gauge punches of NAc were obtained at varying times after the last cocaine or saline injection and frozen on dry ice until further use. Samples were homogenized in TRIzol, purified with RNAeasy Micro columns, reverse transcribed using an iScript Kit, and quantified using SYBR green. Each reaction was performed in duplicate and normalized to glyceraldehyde–3–phosphate dehydrogenase (*Gapdh*) levels, which were not themselves altered by cocaine exposure.

Western blotting

Western blotting was performed as described previously (Sun et al., 2015, 2016). Briefly, frozen bilateral NAc punches from individual mice were homogenized in RIPA buffer, and 50 μg of protein were loaded onto 4–15% gradient Tris–HCl polyacrylamide gels for electrophoresis (Bio–Rad). Proteins were transferred to nitrocellulose membranes, which were then blocked with Odyssey® blocking buffer (Li–Cor, NE, USA), and incubated overnight at 4 °C with primary antibodies (BAZ1A: Bethyl A301–318A) in Odyssey® blocking buffer. The next day, membranes were incubated with IRDye® secondary antibodies (1/5000; Li–Cor, Lincoln, NE, USA; 1 h at room temperature) in between washes with 1× Tris–Buffered Saline plus 0.1% Tween–20. Blots were imaged with the Odyssey® Infrared Imaging system (Li–Cor, Lincoln, NE, USA) and quantified by densitometry using ImageJ (NIH, Bethesda, Maryland, USA). The amount of protein blotted onto each lane was normalized to levels of GAPDH or β-tubulin, neither of which was altered in NAc by cocaine exposure.

Viral–mediated gene transfer

Viral-mediated gene transfer was performed as described previously (Sun et al., 2015, 2016). Briefly, mice were positioned in stereotaxic instruments under ketamine (100 mg/kg)/xylazine (10 mg/kg) anesthesia and thirty–three gauge syringe needles were bilaterally lowered into NAc (anterior/posterior + 1.6; medial/lateral + 1.5; dorsal/ventral − 4.4 mm from Bregma, 10° angle) to infuse 0.5 μl of HSV-GFP or HSV-BAZ1A.

Conditioned place preference (CPP)

CPP was performed as described previously beginning one day after viral surgery (Sun et al., 2016). Briefly, animals were trained in a three-chamber apparatus with two distinct side chambers. After a baseline preference measure, which showed that mice exhibit no pre-existing bias for either side chamber, mice were conditioned for 30 min over 2 days to the saline-paired side in the morning and the cocaine-paired side (7.5 mg/kg i.p.) in the afternoon. On the final day, mice were placed again in the central compartment at noon and allowed to move freely between the two side chambers for 20 min. CPP scores were calculated as time spent in the cocaine-paired chamber minus time spent in the saline-paired chamber. This dose of cocaine was previously determined to reveal both increases and decreases in CPP after experimental manipulations.

Locomotor activity

For the 1st locomotor experiment, mice were injected with saline and habituated in a locomotor recording chamber for 30 min after recovery from stereotaxic surgery (Scobie et al., 2014; Sun et al., 2016). Then, for the next 5 days, locomotor activity was monitored for 30 min immediately after cocaine injections (10 mg/kg i.p.). For the challenge experiment, saline or cocaine (10 mg/kg i.p) was administered daily for seven consecutive days, and animals were placed in the locomotor chamber and monitored for their activity. Following locomotor training, animals were given stereotaxic intra-NAc infusion of either HSV-GFP or -BAZ1A, allowed two days to recover, and then given a challenge injection of saline or cocaine (10 mg/kg i.p) and monitored for their locomotor activity for 30 min. This dose of cocaine was previously determined to reveal both increases and decreases in locomotor activity after experimental manipulations.

Chromatin immunoprecipitation (ChIP), library preparation, and sequencing

Published procedures were utilized (Feng et al., 2014; Sun et al., 2015). For each ChIP–sequencing (ChIP-seq) replicate, bilateral 14–gauge NAc punches were pooled from 5–10 mice, lightly fixed MNase digested, and immunoprecipitated using sheep anti–rabbit magnetic beads (Invitrogen) conjugated to an antibody targeting total H3 (Abcam). Immunoprecipitated DNA and total (input) genomic DNA were prepared for ChIP–seq using an Illumina kit according to the manufacturer's instructions. Each experimental condition was analyzed with independent biological triplicates.

ChIP–seq data analysis

ChIP–seq data alignment and analysis were conducted as described previously (Sun et al., 2015). ChIP-seq data were of sufficient quality and coverage for downstream nucleosome positioning determinations. For analysis of nucleosome position and occupancy, DANPOS was applied in the dynamic analysis of nucleosomes (Chen et al., 2013) with analysis parameters as described previously (Sun et al., 2015).

Statistical analysis

Student's *t*-tests were used whenever two groups were compared, while one–way and two–way ANOVAs were performed to determine significance for all other data.