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Cocaine alters *Homer1* natural antisense transcript in the nucleus accumbens

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ABSTRACT

Natural antisense transcripts (NATs) are an abundant class of long noncoding RNAs that have recently been shown to be key regulators of chromatin dynamics and gene expression in nervous system development and neurological disorders. However, it is currently unclear if NAT-based mechanisms also play a role in drug-induced neuroadaptations. Aberrant regulation of gene expression is one critical factor underlying the long-lasting behavioral abnormalities that characterize substance use disorder, and it is possible that some drug-induced transcriptional responses are mediated, in part, by perturbations in NAT activity. To test this hypothesis, we used an automated algorithm that mines the NCBI AceView transcriptomics database to identify NAT overlapping genes linked to addiction. We found that 22% of the genes examined contain NATs and that expression of *Homer1* natural antisense transcript (*Homer1-AS*) was altered in the nucleus accumbens (NAc) of mice 2 h and 10 days following repeated cocaine administration. In *in vitro* studies, depletion of *Homer1-AS* lead to an increase in the corresponding sense gene expression, indicating a potential regulatory mechanisms of *Homer1* expression by its corresponding antisense transcript. Future *in vivo* studies are needed to definitely determine a role for *Homer1-AS* in cocaine-induced behavioral and molecular adaptations.

1. Introduction

Recent high-throughput sequencing studies have uncovered tens of thousands of RNA transcripts with little or no protein-coding potential (termed noncoding RNAs) (Carninci et al., 2005; Katayama et al., 2005a; Birney et al., 2007). Initially thought of as transcriptional noise, research has now shown that noncoding RNAs have fundamental roles in multiple aspects of cellular physiology (Magistri et al., 2012; Qureshi and Mehler, 2012). Long noncoding RNAs (lncRNAs), defined as noncoding transcripts > 200 nucleotide bases in length, are pervasively expressed across mammalian genomes and have been implicated in several aspects of transcriptional and posttranscriptional regulation (Feng et al., 2006; Rinn et al., 2007; Nowacki et al., 2008; Huarte et al., 2010). LncRNAs are generally classified based on their anatomical properties and their gene loci. For example, natural antisense transcripts (NATs) overlap other genes on the opposite strand of the DNA while intergenic lncRNAs (lincRNAs) are located between genes. Interestingly, many of these lncRNAs show specific spatial and temporal expression patterns within tissues and cells (Clark et al., 2011). In the brain, for example, numerous lncRNAs are selectively expressed in neurons (Mercer et al., 2008; Belgard et al., 2011), indicating that some lncRNAs may be specifically involved in neuronal function (Qureshi and Mehler, 2012).

Natural Antisense Transcripts (NATs) are transcribed from the opposite DNA strand of other RNA transcripts with which they share sequence complementarities (Magistri et al., 2012). Transcriptome-wide analyses by the FANTOM and ENCODE consortia have revealed that up to 66% of protein-coding genes contain NATs (Katayama et al., 2005b; Djebali et al., 2012), indicating their vast prevalence and potential importance in biological mechanisms. Of the proposed functional mechanisms of NATs, regulation of chromatin architecture and epigenetic memory have received much attention, as some antisense transcripts may provide a scaffold by which proteins can interact with DNA and chromatin in a locus-specific manner (Saxena and Carninci, 2011; Magistri et al., 2012). Moreover, many NATs are expressed in the CNS where they have been shown to play fundamental roles in a variety of biological processes. For example, our previous work demonstrates that NAT dysregulation is associated with Alzheimer's disease pathophysiology (Modarresi et al., 2011; Magistri et al., 2016), and in separate reports we have also revealed that NATs contribute to Parkinson's

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disease, Autism, Fragile X mental retardation, seizures and metabolism (Scheele et al., 2007; Faghihi et al., 2010a; Pastori et al., 2014; Hsiao et al., 2016; Silva et al., 2016). Mechanistically, we and others have shown that NATs can mediate epigenetic modification of genes in *cis*, making them unexplored targets to achieve specific up-regulation of gene expression (Modarresi et al., 2012). Additionally, NATs have been found to affect disease-related gene expression by modifying mRNA stability and microRNA function (Faghihi et al., 2010b; Modarresi et al., 2011). Thus, these findings suggest that NATs are dysregulated in multiple neuropathologies and that endogenous gene expression can be specifically regulated by targeting NATs.

With such integral roles in transcriptional and posttranscriptional regulation, it is not surprising that dysregulation of NATs have been linked to multiple diseases (Scheele et al., 2007; Faghihi et al., 2008; Han et al., 2012; Yang et al., 2012), but little is known about their involvement in substance use disorder. Two recent reports, however, indicate that lncRNAs, in general, may play a role in cocaine-induced adaptations, as expression of several lncRNAs was altered in the nucleus accumbens (NAc) and midbrain of post-mortem human cocaine abusers (Michelhaugh et al., 2011; Bannon et al., 2015) and in mice following cocaine conditioned place preference (Bu et al., 2012). To better understand a role for NATs in response to repeated cocaine use, we took advantage of a recently published automated algorithm that mines the AceView transcriptomic database to identify biologically relevant sense-antisense pairs (Velmeshev et al., 2013) and revealed that numerous genes that have been previously associated with cocaine-induced neuroadaptations contain NATs. In *in vivo* studies, we found that expression of *Homer1-AS* was significantly altered in the NAc of mice following repeated cocaine administration, and that siRNA-mediated depletion of *Homer1-AS in vitro* altered corresponding sense gene expression. Together, these data indicate NAT-based mechanisms may contribute to cocaine-mediated molecular adaptations.

2. Methods and materials

2.1. Animals

Male C57BL/6 mice (8–10 weeks old, Charles River Laboratories) were housed 4 animals per cage, under a regular 12 h/12 h light/dark cycle with *ad libitum* access to food and water. Mice were housed in a humidity and temperature-controlled, AAALAC-accredited, animal facility at the University of Miami Miller School of Medicine. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) and conducted according to specifications of the NIH as outlined in the Guide for the Care and Use of Laboratory Animals.

2.2. Drug treatments

Cocaine HCl (NIDA, Research Triangle Park, NC) was dissolved in 0.9% sterile saline. Animals received one intraperitoneal (i.p.) injection of saline or cocaine (20 mg/kg) once a day for 10 consecutive days for repeated exposure experiments and a single injection of saline or cocaine (20 mg/kg) for acute studies. Two hours or 10 days after the last injection, bilateral tissue punches of the nucleus accumbens (NAc) were collected on ice and flash frozen in liquid nitrogen and then processed for RNA analysis as described below. Doses for all drugs were based on their salt form.

2.3. Bioinformatics analysis

We utilized a previously published bioinformatics algorithm to identify Natural Antisense Transcripts (NATs) from AceView transcriptome database (Velmeshev et al., 2013). The open-access algorithm and the manual are available at https://github.com/DmitryVel/NATs. Briefly, the algorithm takes an input list of genes and first identifies all transcripts expressed from the antisense strand of the target locus or the gene promoter (1 kb downstream from the transcription start site). Next, transcripts containing a significant open reading frame according to the annotation on AceView are filtered out to retain noncoding NATs. We utilized this pipeline to investigate the expression of antisense transcripts overlapping with 545 genes. This list was based on previously published genes that have been implicated in cocaine-induced neuroadaptations (Renthal et al., 2009; Maze et al., 2010; Ferguson et al., 2013; Feng et al., 2014) and from the top gene searches on Allan Brain Atlas related to neuroplasticity. The pipeline output yielded the existence of an antisense overlap, the position of the overlap, the NAT Aceview name, the NAT length and the overlap start and stop sites (Table S1). PANTHER (http://www.pantherdb.org/) was used to categorize NAT-containing genes based on protein class.

2.4. Cell culture

Neuro-2a (N2a) cell lines (ATCC) were maintained in Advanced DMEM supplemented with 10% FBS and 1% primocin. In a 6 well plate, ~ 400,000 cells were plated and transfected 24 h later with scrambled control (Qiagen SI03650318) or *Homer1-AS* siRNA (Forward: 5′ GUACAAAUGCAAUGUGUUAUU 3′, Reverse: 5′ UAACACAUUGCAUUU GUACUU 3′) in lipofectamine RNAiMax (Invitrogen) according to the manufacturer's instructions (20 or 40 nM for 48 h). A blank group treated with transfection reagent only was also included as an additional control. After treatment, N2a cells (6 biological replicates with the same passage number and treatment time) were harvested for RNA analysis as described below.

2.5. Quantitative real-time PCR

Following treatment, RNA was isolated using a Trizol and column RNA extraction kit as described by the manufacturer (RNeasy kit, Qiagen). Samples were run in triplicate replicates, normalized to beta-actin and analyzed using the $2^{-\Delta\Delta CT}$ method. NAT primers (Table S1) were preferentially designed to span a spliced junction of the antisense transcript that does not overlap any spliced junction of the sense gene to avoid sense transcript amplification. In cases where no junctional primers could be designed, we designed a primer pair targeting an exon of the antisense transcript and utilized a strand-specific qRT-PCR to amplify only the antisense transcript. To perform strand-specific measurement of antisense transcript expression, we designed primers for a region of antisense transcript that overlaps with an intron or the promoter of the sense gene. Using the one-step RNA-to-Ct SYBR Green Kit (Life Technologies, 4,389,986), the reverse transcription (RT) step was performed in a 384-well optical plate using reverse primers to specifically reverse-transcribe antisense RNA and to exclude the possibility of measuring the expression of the sense pre-mRNA. Samples were then incubated at 95 °C for 5 min to inactivate the reverse transcriptase enzyme. Forward primers were then added to the reaction and quantitative PCR was performed on the same plate. We included no-RT control and no-template controls for each set of primers to control for non-specific binding and melting curves were analyzed to verify PCR specificity. *Homer1* and *beta-actin* expression were measured with Taqman assays as described by the manufacturer (ThermoFisher, Mm00516275_m1 and Mm02619580_g1). A map of the sense/antisense overlap of *Homer1/Homer1-AS* may be found on the Aceview mouse database (https://www.ncbi.nlm.nih.gov/ieb/research/acembly/) by searching for *mahemo*. Using SnapGene software, an additional map of *Homer1* sense and antisense is shown in Fig. 4. In Figs. 2, 3 and 5, the spliced form of *Homer1-AS* was measured.

2.6. Data analysis

Graphpad Prism software was used for graph preparation and statistical analysis. Rq values were normalized to saline or vehicle-treated

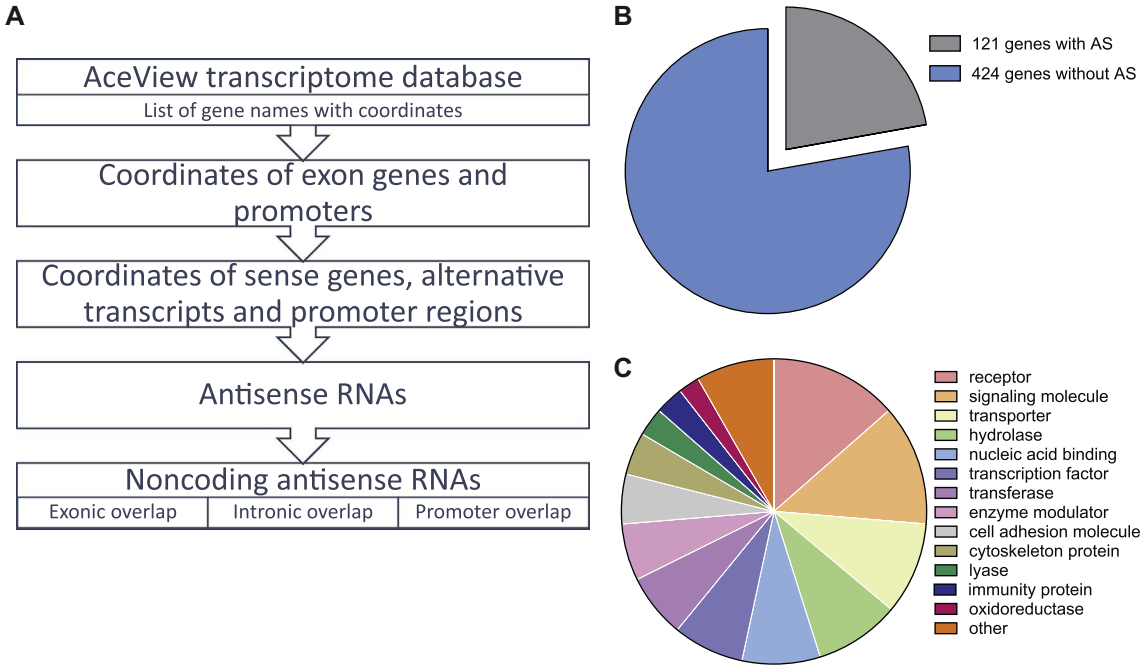

Fig. 1. Bioinformatics pipeline and classification of noncoding antisense RNAs. (A) Schematic of bioinformatics pipeline used to identify natural antisense transcripts. (B) One hundred and twenty-one noncoding antisense (AS) RNAs were identified from 545 genes examined. (C) Gene ontology analysis of NAT-containing genes based on protein class.

samples and compared between groups using one- or two-way ANOVA. Newman-Keuls, Bonferroni or Benjamini and Hochberg false discovery rate test was performed for *post-hoc* comparisons. Data are mean ± SEM, and the level of significance was set to ≤ 0.05.

3. Results

To explore a potential role for NATs in response to repeated cocaine exposure, we compiled a list of 545 genes that have been associated with neuroplasticity and/or addiction, and used our previously described algorithm to mine the AceView database to identify overlapping sense-antisense pairs (Velmeshev et al., 2013) (Table S1). Using this pipeline (Fig. 1A), we found that 22% (121/545) of the genes examined contained NATs, several of which (23/121) contained two or more NATs per gene (Fig. 1B). Gene ontologies were diverse, with the largest proportion of the NAT-containing genes classified under receptor and signaling molecules (Fig. 1C). The vast majority of these antisense RNAs were classified as intronic overlap with respect to the sense gene, though some were found to overlap with the promoter and exonic regions (Table S1).

To determine if the expression of these transcripts was altered in response to cocaine, we selected 25 antisense RNAs, many of which overlap with genes that have been implicated in cocaine-induced adaptations (Table 1), and quantified their expression in the nucleus accumbens (NAc) 2 h following the last cocaine or saline injection. A two-way ANOVA showed significant interaction between antisense transcript and treatment ($F_{(24,\ 394)} = 3.0$, $P < 0.0001$), a main effect for antisense transcript ($F_{(24,\ 394)} = 3.0$, $P < 0.0001$, $n = 7–9$) but no main effect for treatment ($F_{(1,\ 394)} = 0.6$, $P > 0.05$) (Fig. 2). Benjamini and Hochberg *post hoc* test revealed that *Homer1-AS* was significantly altered following repeated cocaine treatment ($P < 0.0005$, Fig. 2, Table S2). Although *Homer1-AS* was increased in the NAc following repeated cocaine administration, no change in *Homer1* expression was observed at the same time point following repeated cocaine injections (Fig. 3A). *Homer1-AS* remained significantly elevated 10 days following repeated cocaine injections ($P < 0.005$, Fig. 3B). In additional experiments, an acute injection of cocaine increased *Homer1*, but not *Homer-AS*, expression in the NAc ($P < 0.0005$, Fig. 3C). A schematic of the sense-antisense overlap between *Homer1/Homer1-AS* is shown in Fig. 4.

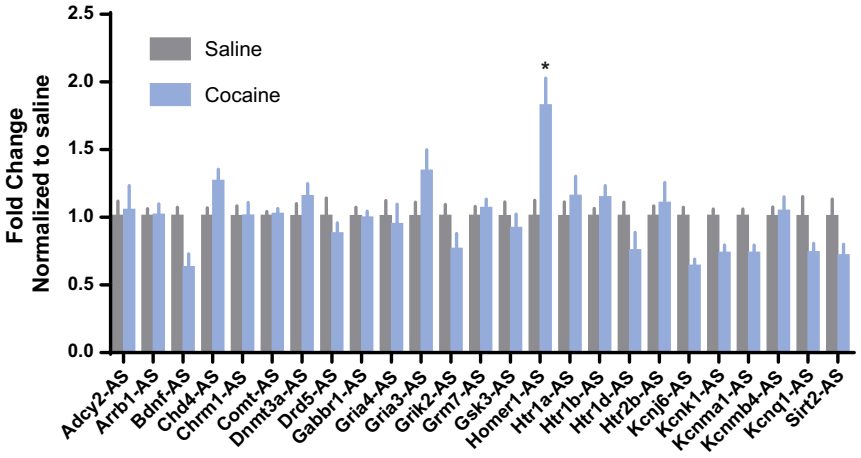

Fig. 2. Expression of noncoding antisense RNAs in the NAc. Following repeated cocaine or saline injections, NAc was collected for qPCR analysis. Significant change in *Homer1-AS* expression was revealed in cocaine-treated mice. *$P < 0.0001$ compared to saline *via* Benjamini and Hochberg false discovery rate test, $n = 7–9$.

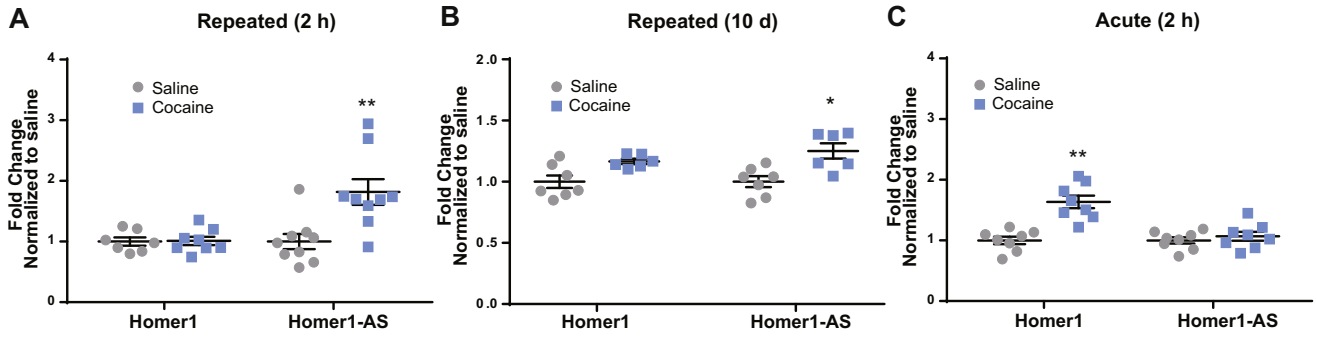

Fig. 3. *Homer1* and *Homer1-AS* expression following repeated or acute cocaine injections. Repeated cocaine injections increased *Homer1-AS*, but not *Homer1*, in the NAc 2 h (A) and 10 days (B) after the last injection. C) Increased *Homer1*, but not *Homer1-AS*, expression in the NAc 2 h following a single injection of cocaine. $^{*}P < 0.005$ and $^{**}P < 0.0005$ compared to saline *via* Bonferroni post-hoc test, $n = 6–9$.

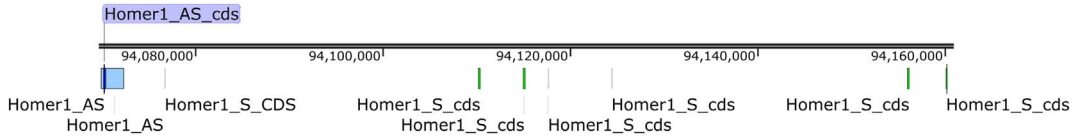

Fig. 4. Graphical representation of *Homer1* sense and antisense map. Genomic location of mouse antisense (blue) and sense (green) RNA pairs for *Homer1*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

NATs typically employ *cis*-regulatory mechanisms to positively (concordant) or negatively (discordant) regulate the expression of their overlapping sense mRNA (Magistri et al., 2012). Though *Homer1* is known to be altered in the NAc following cocaine administration (Szumlinski et al., 2008), it is unclear if *Homer1-AS* modulates its expression. To identify the relationship between *Homer1/Homer-AS* expression, we utilized siRNA-mediated knockdown to significantly deplete *Homer1-AS* in N2a cells ($F_{(3,\ 20)} = 8.1$, $P < 0.005$, $n = 6$) (Fig. 5A). *Homer1-AS* knockdown resulted in a discordant regulation of *Homer1* ($F_{(3,\ 20)} = 60.7$, $P < 0.0001$, n = 6) (Fig. 5B), indicating a potential regulatory mechanisms between the protein-coding gene by its corresponding antisense transcript.

4. Discussion

Recent transcriptomic studies by FANTOM, GENCODE and ENCODE consortia have revealed that RNA plays a variety of roles in nuclear architecture, X chromosome inactivation, imprinting, DNA looping, transcription, translation and epigenetic processes (Magistri et al., 2012). While small noncoding RNAs such as microRNAs have been shown to be important in regulating addiction-relevant neuroplasticity in the brain and cocaine-seeking behaviors (Bali and Kenny, 2013), little is known about the involvement of long noncoding RNAs, particularly natural antisense transcripts (NATs), in cocaine-induced neuroadaptations and behavior. Our results show that numerous genes contain NATs and reveal that *Homer1-AS* was altered in the nucleus accumbens following repeated cocaine administration. Thus, these data, along with previous reports, indicate that NAT-based mechanisms may regulate transcriptional and/or post-transcriptional responses to cocaine and contribute to cocaine-induced neuroadaptations.

Although cocaine-mediated regulation of *Homer1* is an important adaptation underlying drug-seeking behaviors, the effects of cocaine on *Homer1* expression are quite complex and dependent upon the duration of cocaine administration, length of withdrawal, and the brain region/isoform examined (Szumlinski et al., 2008). For example, acute cocaine administration transiently increases mRNA and protein of the immediate early gene, Homer1a, and the constitutively expressed isoform, Homer1b/c, in the NAc (Brakeman et al., 1997; Fourgeaud et al., 2004;

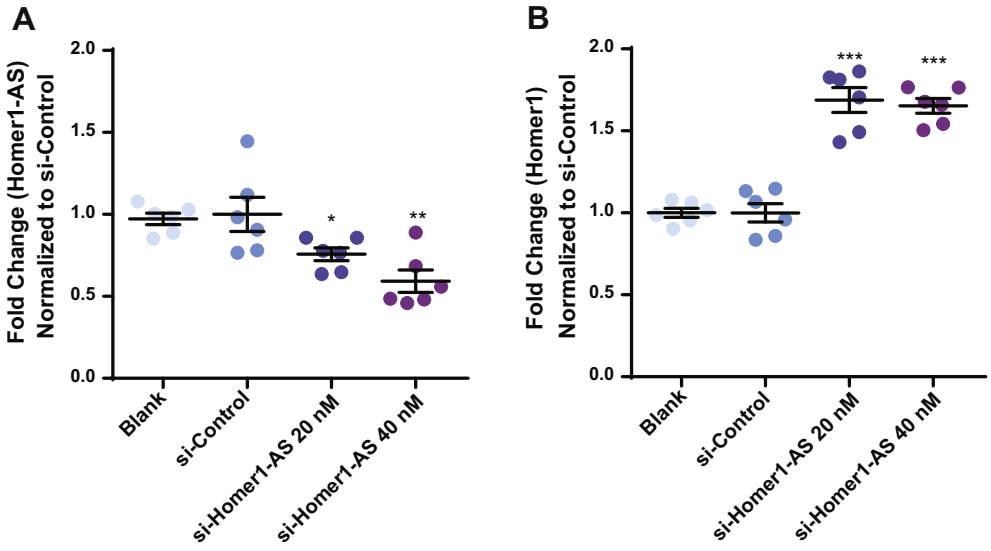

Fig. 5. Sense and antisense expression following *Homer1-AS* knockdown. A) Reduction of Homer1-AS following siRNA treatment in N2a cells. B) Increase in *Homer1* expression following *Homer1-AS* knockdown in N2a cells. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$ compared to si-Control *via* Newman-Keuls *post-hoc* test.

Table 1
Antisense RNAs examined in the mouse Nac.

Gene name	NAT name	Interaction type	Overlap with sense
Adenylate cyclase 2	*Adcy2-AS*	intronic	69,137,671–69,138,140
Beta arrestin 1	*Arrb1-AS*	intronic	106,751,848–106,751,978
Brain-derived neurotrophic factor	*Bdnf-AS*	promoter	109,513,857–109,514,010
Chromodomain helicase DNA binding protein 4	*Chd4-AS*	intronic	125,078,861–125,078,948
Cholinergic receptor, muscarinic 1B, CNS	*Chrm1-AS*	promoter	8,737,279–8,737,826
Catechol-*O*-methyltransferase	*Comt-AS*	intronic	18,421,262–18,421,605
DNA (Cytosine-5-)-methyltransferase 3 Alpha	*Dnmt3a-AS*	intronic	3,884,675–3,884,792
Dopamine receptor 5	*Drd5-AS*	intronic	38,712,870–38,713,757
GABA receptor 1	*Gabbr1-AS*	intronic	37,169,668–37,169,798
Glutamate receptor, AMPA 4, ionotropic	*Gria4-AS*	intronic	4,514,665–4,514,849
Glutamate receptor, AMPA 4, ionotropic	*Gria3-AS*	intronic	39,016,690–39,017,360
Glutamate kainate receptor 2	*Grik2-AS*	intronic	48,863,035–48,865,786
Glutamate Metabotropic Receptor 7	*Grm7-AS*	exonic	110,595,506–110,595,915
Glycogen synthase kinase 3	*Gsk3-AS*	intronic	38,089,077–38,089,347
Homer homolog 1	*Homer1-AS*	intronic	94,073,408–94,073,537
5-HT1a receptor	*Htr1a-AS*	intronic	106,236,748–106,238,201
5-HT receptor 1B	*Htr1b-AS*	intronic	81,525,537–81,525,886
5-HT receptor 1D	*Htr1d-AS*	intronic	135,990,295–135,990,924
5-HT2b receptor	*Htr2b-AS*	promoter	88,009,163–88,009,546
Potassium inwardly-rectifying channel, subfamily J, member 6	*Kcnj6-AS*	exonic	94,970,290–94,970,498
Potassium channel, subfamily K, member 1	*Kcnk1-AS*	exonic	128,553,148–128,553,570
K large conductance Ca-activated channel, subfamily M, a1	*Kcnma1-AS*	intronic	24,824,213–24,825,059
K large conductance Ca-activated channel, subfamily M, b4	*Kcnmb4-AS*	exonic	115,911,266–115,911,408
Potassium voltage-gated channel, subfamily Q, member 1	*Kcnq1-AS*	intronic	150,399,016–150,403,528
Sirtuin 2	*Sirt2-AS*	intronic	29,566,257–29,566,534

Zhang et al., 2007). Following 3 weeks of withdrawal from repeated cocaine injections, Homer1b/c protein is decreased in the NAc, which also coincides with reductions in mGluR5 protein and manifestation of cocaine-induced behavioral sensitization (Swanson et al., 2001). In functional studies, knockout or oligo-induced depletion of *Homer1* in the NAc enhances locomotor responses to cocaine, whereas over-expression of the constitutive isoform prevents cocaine sensitization (Ghasemzadeh et al., 2003; Szumlinski et al., 2004). The data presented here add another layer of complexity to the regulation of *Homer1* in response to cocaine. We identified a noncoding antisense RNA that overlaps with *Homer1* and whose expression is increased in the NAc in response to repeated, but not acute, cocaine injections. Although we revealed that acute cocaine transiently increases *Homer1* in the NAc, we did not observe a change in *Homer1* mRNA expression 2 h after repeated cocaine administration. These data are consistent with previous studies that have shown a temporary increase in Homer1 mRNA and protein following an acute cocaine injection (Brakeman et al., 1997; Fourgeaud et al., 2004; Zhang et al., 2007) and a delayed decrease in Homer1 protein following repeated cocaine injections (Swanson et al., 2001). Thus, based on the differences in temporal regulation of *Homer1* and *Homer1-AS,* it is conceivable that *Homer1-AS* affects *Homer1* protein expression in the NAc *via* post-transcriptional processes. In *in vitro* studies, we revealed that siRNA-mediated knockdown of *Homer1-AS* increased *Homer1* mRNA expression, indicating a discordant regulation mechanism between *Homer1* sense and antisense expression. Although we have previously shown that NAT knockdown produced a similar upregulation of sense gene expression in both *in vivo* and *in vitro* experiments (Modarresi et al., 2012), it is possible that *Homer1/Homer-AS* interact differently in specific cells types and under *in vitro vs. in vivo* conditions. Thus, future *in vivo* studies are needed to determine a mechanistic role for *Homer1-AS*. Nonetheless, given that cocaine increases *Homer1-AS* and that changes in *Homer1* contribute to the long-lasting neurochemical and behavioral abnormalities that characterize addiction (Szumlinski et al., 2008), antisense transcripts that overlap with *Homer1* may play an important role in cocaine-induced neuroadaptations.

Our earlier findings indicate that the expression of *Bdnf*, a gene importantly involved in the development of cocaine-induced neuroplasticity and behaviors (Li and Wolf, 2014), is regulated by a *Bdnf* antisense transcript (Modarresi et al., 2012). Interestingly, in our current studies, *Bdnf-AS* was reduced 38% following repeated cocaine exposure, though this reduction was not statistically significant when adjusted for multiple comparisons. Due to the stringency of the Benjamini and Hochberg false discovery rate test, Type II errors (false negatives) are possible and future studies are needed to address the functional role of *Bdnf-AS* in response to cocaine. However, given that *Bdnf* is known to increase in the NAc following cocaine exposure and self-administration (Graham et al., 2007), it is possible that *Bdnf* and *Bdnf-AS* also interact in a discordant manner to regulate behavioral and molecular responses to drugs of abuse. Indeed, a recent study revealed a correlation between polymorphisms in *BDNF-AS* and heroin abuse in the Han Chinese population (Jin et al., 2016), indicating the potential clinical importance of *BDNF-AS* in substance use disorders. Likewise, the antisense transcript of *Kcnq1* (referred to as *Kcnq1ot1*), which was reduced by 35% in the NAc by cocaine, has been studied extensively and is known to silence multiple genes in the *Kcnq1* domain by establishing a repressive higher order chromatin structure (Mohammad et al., 2009) and, similar to our results, is altered in the mouse NAc by methamphetamine (Zhu et al., 2015). Thus, the epigenetic mechanisms by which *Kcnq1-AS* and other NATs regulate transcriptional and behavioral responses to psychostimulants merit further investigation.

Utilizing microarray analysis, previous studies have also identified several long noncoding RNAs that were altered in the NAc and midbrain dopamine neurons of human cocaine and heroin abusers (Michelhaugh et al., 2011; Bannon et al., 2015) and in the NAc of mice following cocaine conditioned place preference (Bu et al., 2012). Although NATs were not the primary focus of these previous studies, several of the long noncoding RNAs that were altered by cocaine were antisense transcripts (52/603 in the mouse NAc and 16/32 in the human midbrain) (Bu et al., 2012; Bannon et al., 2015). To our knowledge, *Homer1-AS* and other NATs examined in the current study were not investigated in previous cocaine studies. However, our current results and previous studies are far from exhaustive. Subsequent RNA-seq studies utilizing the latest lncRNA annotations are needed in order to have comprehensive understanding of the NATs involved in substance use disorder. Another interesting observation in the current study is that few NATs were significantly altered in the NAc by repeated cocaine injections. The stringency of the Benjamini and Hochberg false

discovery rate test likely accounts for why only one of the NATs examined met statistical significance. Although only a portion of lncRNAs that were significantly altered by drugs of abuse were NATs in the above studies, it is important to note that many NATs are expressed at low levels and in a cell type-specific manner (Cabili et al., 2011; Derrien et al., 2012; Li et al., 2013; Dong et al., 2015). Thus, small changes in expression that are observed in tissue homogenate may have functional importance in specific cell types. In fact, recent FANTOM5 analyses indicate the potential functionality of 69% of lncRNAs (Hon et al., 2017). Future studies examining brain region-, cell type- and temporal-specific expression profiles of noncoding antisense RNAs following cocaine use, along with functional experiments examining the behavioral consequences of NAT manipulation on drug-seeking behaviors, will lead to a better understanding of NATs in drug-induced neurobehavioral adaptations.

In summary, imbalanced expression of NATs may result in the silencing or activation of partner protein-coding genes, thus providing a potentially interesting and novel mechanism to explain the aberrant gene regulation following repeated drug use. Based on these initial findings and previously published data, we hypothesize that repeated cocaine administration leads to a dysregulation of natural antisense transcripts that may act in concert with transcriptional and post-transcriptional machinery to pathologically alter gene expression and behavior. However, additional studies are needed to better understand the anatomical and temporal dynamics of NAT expression following acute and repeated cocaine exposure and to reveal the functional importance of specific NATs during drug-seeking behaviors. Such experiments may lead to the incorporation of NATs into epigenetic models of drug-induced plasticity and open the door for critically-needed new therapeutic avenues for substance use disorder.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mcn.2017.10.003.

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