

NESTLER LAB

# SHADOW “SFN” ABSTRACTS SUMMER/FALL 2020

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## Transcriptional Reprogramming of Brain Reward Circuitry by Heroin Self-Administration and Relapse

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Opioid addiction exacts a devastating toll on individuals, their families, and the healthcare system. A major hurdle in treating opioid addiction is the chronic susceptibility to relapse faced by users, often triggered by re-exposure to drug or cues previously associated with drug intake. The persistence of opioid-induced behavioral abnormalities is mediated in part by changes in gene expression programs within interconnected regions of the brain involved in reward processing. Although previous studies have identified candidate genes regulated by opioids, no studies have comprehensively examined transcriptome-wide changes across the reward circuitry after volitional opioid intake. Here, we combine heroin self-administration in mice, molecular profiling by RNA sequencing (RNA-seq), and bioinformatics approaches to identify novel gene networks throughout brain reward regions altered by opioid use or relapse. First, mice underwent 15 daily 4 hr self-administration sessions wherein lever pressing lead to intravenous delivery of saline or heroin (FR1, 0.05 mg/kg/inf). Mice were then euthanized either 24 hr after the last session or after a 30-day withdrawal period. In the 30-day group, mice received either a saline or heroin challenge (1 mg/kg) and were placed back into self-administration chambers for a 2 hr drug-seeking test, after which mice were euthanized immediately. This design enabled comparisons of multiple addiction-relevant outcomes, including first-ever heroin exposure, early withdrawal from chronic use, context-induced drug-seeking, and combined drug+context re-exposure. RNA-seq was performed on six interconnected brain reward regions: prefrontal cortex, nucleus accumbens, dorsal striatum, basolateral amygdala, ventral hippocampus, and ventral tegmental area. Using this approach, we have uncovered numerous changes to gene expression networks throughout the reward circuitry, with many similar and distinct patterns observed across brain regions. Further, we have employed exploratory factor analysis to link gene expression changes in individual mice with their behavioral profiles indicative of an addiction-like state, and identified several region-specific patterns of gene priming by each re-exposure condition. We are now using gene co-expression network analysis to determine key drivers of these transcriptional changes that promote susceptibility to relapse. These studies provide fundamental insights into opioid-induced transcriptional regulation, and will yield important intervention targets to reverse behavioral abnormalities underlying relapse to drug-seeking.

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## Epigenetic-Mediated Paternal Transmission of Stress Phenotypes to Offspring

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Depression risk has long been known to be highly influenced by both genetic and environmental factors. More recently, it has been proposed that epigenetic mechanisms may also contribute, representing a third basis of risk. Studies on intergenerational trauma in rodents show that the offspring of males that have been exposed to stress show anxiety- and depression-like phenotypes. We tested the hypothesis that transcriptional alterations in sperm during chronic social defeat stress (CSDS) in mice are responsible for transmitting part of the increased susceptibility to stress phenotypes to the F1 generation. We found F1 offspring of defeated fathers categorized as either resilient or susceptible to CSDS show altered stress sensitivity phenotypes. However, susceptible fathers persistently transmit stress phenotypes in both male and female offspring, whereas resilient fathers persistently transmit stress phenotypes only to female offspring. Importantly, artificial insemination reveals that sperm mediates some aspects of the behavioral phenotypes seen in the offspring. To better understand how the transcriptome of sperm is altered by CSDS we used RNA-sequencing and reveal both preexisting and CSDS-induced differences in the sperm transcriptome of both resilient and susceptible animals versus normal controls. In susceptible males, there was a particularly prominent increase in differentially expressed long non-coding RNA (lncRNA) genes following CSDS, which correlation analysis revealed was accompanied by a loss of regulation of protein-coding genes by lncRNAs in sperm of susceptible males. This work provides novel evidence for lncRNA involvement in mediating the paternal transmission of stress phenotypes in a susceptible-specific manner, and contributes to our understanding of the molecular basis by which paternal stress transmits changes in stress susceptibility to offspring in part through epigenetic mechanisms.

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## Cell Type-Specific Dopamine Signaling in Ventral Hippocampus Tracks Anxiety to Trigger Relapse to Drug Seeking

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Relapse to drug seeking and taking in abstinent individuals is one of the most clinically devastating hallmarks of drug addiction, and is thought to result from long-lasting alterations in the interconnected brain regions that integrate midbrain dopamine signals that normally guide reward-related learning. Among those, surprisingly little is known about the specific circuit and molecular maladaptations that occur in ventral hippocampus (vHipp), despite its well-established role in controlling anxiety- and relapse-like behaviors. To address these shortcomings, we here study dopamine release and dopaminergic neurons in mouse vHipp – which can be segregated based on their expression of either the dopamine D1 or D2 receptor (as done routinely for striatum) – to propose a model of dopamine action in vHipp under baseline and drug-induced conditions. First, using fluorescent probes for *in vivo* dopamine sensing during anxiety testing, we showed that anxiogenic environments trigger higher dopamine release in vHipp. Conversely, cocaine-paired contextual cues elicited a decrease in vHipp dopamine, suggesting anxiolytic effects with conditioning. We then assessed the postsynaptic consequences of changes in dopamine levels by coupling dopamine imaging with fiber photometry recordings of D1- or D2-expressing vHipp cell activity in the same behavioral settings. At the histological level, D1- and D2-expressing neurons exhibit a precise topographical organization across vHipp subfields and cell types, consistent with their proposed role in integrating complex and diverse dopamine signals. We are using RNA-sequencing of single, sorted nuclei from D1- and D2-neurons to further dissect these cell types and characterize the transcriptional plasticity mechanisms specifically engaged in response to cocaine exposure. Finally, bidirectional chemogenetic manipulation of D1- or D2-cell activity causally demonstrated their distinct roles in mediating anxiety behaviors, contextual reward learning, and stress- or cocaine-induced reinstatement of extinguished cocaine seeking. Together, we show that drug exposure modifies dopamine dynamics in vHipp, which bidirectionally tracks anxiety levels and differently affects vHipp D1- and D2-expressing neurons. These cell types in turn mediate opposite anxiety responses that differentially control relapse to drug seeking. This work paves the way for further studies of dopamine signal processing in limbic regions, and underscores the complexity of drug-evoked alterations to reward-learning mechanisms.

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## Elucidation of The Astrocyte-Specific Transcriptome Following Exposure to Cocaine

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Drug addiction represents an enormous healthcare burden. To better understand the biological underpinnings, investigations of the transcriptional response to drugs of abuse have demonstrated lasting changes in gene expression throughout the brain's reward circuitry. Historically focused on neurons, emerging evidence increasingly indicates that astrocytes are also involved in disorders of the nervous system, including addiction. Indeed, candidate genes in astrocytes have been identified and, furthermore, manipulation of astrocyte function has been demonstrated to influence rodent behavioral responses to cocaine administration. However, the astrocyte-specific transcriptome following exposure to drugs of abuse has not been investigated. As an initial approach, we examined previously published work (Walker et al., *Biol. Psychiatry*, 2018) and identified numerous astrocyte-enriched genes within RNA-sequencing datasets from bulk homogenates of several brain reward regions that are associated with behavioral responses to cocaine self-administration. Therefore, to obtain more comprehensive and selective data, we utilized whole cell sorting of astrocytes and RNA-sequencing to investigate the astrocyte-specific transcriptome in several key brain regions involved in reward-processing, including the nucleus accumbens and prefrontal cortex, following exposure to cocaine. Subsequent gene ontology analysis revealed a variety of pathways, including synaptic regulation, calcium signaling, and GPCR signaling in both brain regions as being prominently regulated by cocaine exposure. Additional analysis revealed several deduced upstream regulators of this abnormal transcription, such as HTT, CREB1, and several members of the STAT family. Current studies are directed at extending our findings utilizing cocaine self-administration in mice to establish the astrocyte transcriptome in response to drugs of abuse and to then study the role of specific transcripts in contributing to the pathophysiology of addiction.

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## E2f3a Transcription Factor Differentially Regulates Drug-Rewarding Behaviors in a Drug, Sex and Cell-Specific Manner

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The development of drug addiction is characterized by epigenetic changes in the brain reward circuit, leading to the transition from recreational drug use to drug dependence. Most of these epigenetic processes, such as histone modifications, chromatin remodeling and alterations in the expression of transcription factors, have been well characterized in the nucleus accumbens (NAc). Our group recently identified the transcription factor E2F3 as a novel upstream regulator of cocaine-induced gene expression in the NAc. Consistent with this, we demonstrated that viral manipulations of the E2F3a isoform in NAc, but not of E2F3b, bidirectionally control cocaine-induced locomotion and conditioned place preference (CPP) in adult male mice. Interestingly, cell-specific analyses revealed that E2F3a is enriched in D1 receptor-expressing medium spiny neurons (D1-MSNs), where it acts as a key upstream regulator of *Fosb*. *Fosb* in turn encodes its truncated product,  $\Delta$ FosB, that is a critical regulator of transcriptional changes associated with addiction. All of these studies to date have been performed in male mice only. Here, we used viral-mediated gene transfer combined with CPP to assess whether E2F3a differently regulates drug-rewarding behaviors depending on: 1) sex, 2) MSN cell type and 3) type of drug. Our results show that selective E2F3a overexpression in D1-MSNs increased cocaine CPP in female mice, whereas E2F3a overexpression in D2-MSNs has no effect. In contrast, current experiments in male mice preliminarily suggest that E2F3a overexpression in D1-MSNs (but not D2-MSNs) reduces cocaine CPP. Unexpectedly, morphine CPP experiments revealed that E2F3a does not impact opioid rewarding behaviors in either females or males. We are now studying the downstream transcriptional changes upon E2F3a overexpression or knockdown selectively in D1- or D2-MSNs in the context of cocaine exposure. Together, our results highlight E2F3a as a novel regulator of cocaine-elicited transcriptomic modifications in D1-MSNs with potential sex-specific effects.

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## Epigenetic Priming Underlies Aberrant Gene Expression Linked to Cocaine Relapse

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Substance use disorder represents a significant public health crisis with tremendous psychological and financial costs to patients, their families, and society at large. An ongoing focus of research into the molecular pathology of drug addiction is the exploration of mechanisms that preserve altered patterns of gene regulation in a central brain region of reward, the nucleus accumbens (NAc). Highly stable, near-permanent, changes in the epigenetic landscape are believed to underlie the altered transcriptional states in this brain region, which persist despite long-term withdrawal from the drug. However, there is to date no direct link between drug-induced epigenetic marks and aberrant gene expression programs driving relapse. A fundamental challenge is to determine which neuronal subtypes are responsible: the NAc is primarily composed of two opposing types of medium spiny neurons (MSNs), the D1 and D2 dopamine receptor-expressing subtypes, which exhibit dramatic differences in activity and effects on drug reward. In these distinct D1 and D2 MSN subtypes, we investigated how chronic cocaine modifies chromatin structure genome-wide and characterized immediate versus persistent changes in gene regulation. Using fluorescence-activated nuclei sorting (FANS) coupled to ATAC-seq, we surveyed circuit-specific chromatin accessibility in combination with unbiased histone modification profiling by mass spectrometry and ChIP-sequencing. We discovered that chronic cocaine persistently alters NAc chromatin structure, especially in D1 MSNs, involving dramatic depletion of the histone variant H2A.Z, a recently identified memory suppressor, at key neuronal genes. Curiously, genome accessibility in D1 MSNs is prominently increased at these genes even after prolonged withdrawal, and is linked to enduring dysregulation of gene expression upon relapse. Our mass spec approach also revealed that H3K79me2, a relatively unexplored histone mark involved in gene activation, is enriched after prolonged cocaine withdrawal. The histone methyltransferase DOT1L is the only known enzyme to catalyze H3K79me2, and we demonstrate, using validated viral vectors in D1-Cre transgenic mice, that D1 MSN-selective knockdown of DOT1L effectively blocks cocaine conditioned place preference (CPP). Likewise, cocaine CPP is also suppressed when DOT1L catalytic activity is transiently blocked with the small molecule inhibitor pinometostat given systemically, confirming the significance of this new epigenetic pathway in cocaine-related learning and behavior. Together, our studies investigate an emerging view of epigenetic adaptation that may contribute to drug addiction, providing novel insight into circuit-specific epigenetic priming as an important mechanism whereby drugs of abuse alter gene expression and behavior in lasting ways. Since these epigenetic aberrations may be reversible, advancing our mechanistic understanding of such chromatin ‘scarring’ by drugs of abuse could pave the way to new epigenetic therapeutics for substance use disorder as illustrated by our preliminary work with pinometostat.

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## Astrocyte-Specific Expression of the Extracellular Matrix Gene *Htra1* Regulates Susceptibility to Stress in a Sex-specific Manner

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Major Depressive Disorder (MDD) is a highly debilitating and costly mood disorder recently identified as the leading cause of disability worldwide. While the underlying pathophysiology remains incompletely understood, convergent evidence from preclinical and clinical research supports the notion that MDD is related to impaired structural plasticity in key limbic brain regions. Yet, how these structural brain abnormalities contribute to MDD pathology is unclear. The extracellular matrix (ECM) of the brain represents a novel domain for study as it not only provides structural support, but is intimately involved in regulating synaptic plasticity and remodeling as well. We hypothesized that alterations to this complex network of glycosaminoglycans and proteins surrounding neurons and glial cells likely regulate morphological processes possibly involved in the pathophysiology of MDD. To that end, we analyzed transcriptional profiles of ECM-related genes from the nucleus accumbens (NAc) in postmortem brain tissue of humans with MDD as well as in mice exhibiting a depression-like phenotype after exposure to chronic variable stress (CVS). A large number of ECM-specific genes were identified as being differentially expressed, however, only those that were similarly dysregulated across species were selected for downstream manipulations. In particular, we identified *Htra1*, an astrocyte-enriched secreted serine protease, as being significantly down-regulated in males and up-regulated in females by depression/chronic stress across species. We found that selective manipulation of the *Htra1* gene in astrocytes selectively within the mouse NAc increases susceptibility to stress in a sex-specific manner. Taken together, our findings reveal a pivotal role of astroglia as well as the brain's ECM in mediating stress vulnerability differentially in males versus females.

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## Molecular Characterization of Cocaine-Activated Neuronal Ensembles in the Nucleus Accumbens

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Pathological motivation for drug-seeking and consumption, along with the high risk of relapse observed after long periods of withdrawal, are thought to result from persistent adaptations in brain circuits involved in reward learning. In the nucleus accumbens (NAc), drug exposure profoundly and durably affects neuronal physiology within specific neuronal networks involved in the encoding of drug-associated memories. Across cell types, the activation level of individual NAc neurons can be used to further segregate neuronal ensembles selectively recruited during distinct phases of drug exposure, withdrawal, and relapse. The identification and molecular characterization of these specific subsets of neurons is critical to deepen our understanding of how drugs of abuse reshape neuronal networks. The immediate early gene *Arc* is a highly reliable marker of activity used to define neuronal populations recruited during learning processes. In the NAc, *Arc* is induced by cocaine and stands as a pertinent tool to identify drug-activated neurons. We use the tamoxifen-inducible *ArcCreER<sup>T2</sup>* mice (Denny et al., PMID: [24991962](#)), which allow for the stable expression of fluorescent reporters in *Arc*-positive cells, to permanently label neuronal populations activated by cocaine. Long-term tracking of cocaine-activated cells reveals an overlap between ensembles recruited at different time points following cocaine exposure, with their reactivation correlating with behavioral responses. Then, we isolate these tagged cells or their nuclei with Fluorescence Activated Cell Sorting (FACS) and perform single-cell RNA sequencing to phenotype the cell types recruited during distinct phases of cocaine exposure, as well as to characterize the transcriptional signature specific to activated cells. Together, this study employs new methods to advance our understanding of the neuronal processes engaged in subsets of neurons that are specifically recruited during and after exposure to cocaine as well as relapse.

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## Phosphodiesterase 1b is an Upstream Regulator of a Key Gene Network in Nucleus Accumbens Associated With Addiction-Like Behaviors

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Cocaine use disorder (CUD) is a highly prevalent neuropsychiatric disease characterized by compulsive drug taking and repeated relapse. Despite the public health burden of CUD, there remains no FDA-approved medications for this brain disease. Identifying effective treatments for CUD is hindered by an incomplete understanding of which genes are most important in mediating addiction pathophysiology. In this study, we performed unbiased transcriptional network analysis on a published RNA sequencing (RNA-seq) dataset from 6 different brain regions of animals that underwent cocaine self-administration followed by prolonged (30 d) withdrawal plus relapse to identify gene networks associated with individual differences in addiction-like behavior. We ranked modules by their fold enrichment in genes whose expression is significantly correlated with an “addiction index” (AI), a composite score developed by machine learning to capture maladaptive, addiction-like behaviors during cocaine self-administration. We identify phosphodiesterase 1b (*Pde1b*), a  $Ca^{2+}$ /calmodulin-dependent enzyme that catalyzes the hydrolysis of cAMP and cGMP, as the strongest hub gene in module 504 (arbitrary number) in nucleus accumbens (NAc), the module with the strongest association with the AI of all gene modules in this brain region. To investigate the role of *Pde1b* in modulating addiction pathophysiology, we will pair clustered regularly interspaced short palindromic repeats (CRISPR)-based transcriptional regulation tools with viral delivery methods to selectively regulate *Pde1b* expression in the NAc. Our ongoing studies seek to validate that the CRISPR tools achieve robust and selective *Pde1b* regulation *in vitro* and *in vivo*. In future studies, we will investigate the effects of *Pde1b* regulation on addiction-like behaviors in mouse models of CUD. Given successful drug discovery efforts focused on other PDE isoforms, this work raises a novel therapeutic approach for this illness.

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## Transcriptional Profiles of Treatment Resistant Depression in Mouse Models

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Major Depressive Disorder (MDD) is the most prevalent psychiatric disorder worldwide, representing a high level of global economic burden. Despite decades of research, the treatments for MDD remain inadequate for roughly half of patients. Fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI), has been widely used to treat MDD, nonetheless, a majority of patients do not achieve full remission. Further, a subset of those afflicted is considered non-responsive to any orally-available treatments, which is termed treatment-resistant depression (TRD). Ketamine (KET), an antagonist of the glutamate N-methyl-D-aspartate (NMDA) receptor among several other actions, has been shown to induce a rapid antidepressant response in ~50% of TRD patients, thus providing a novel therapeutic approach. However, the molecular mechanisms underlying TRD and subsequent response vs. non-response to KET are poorly understood, thus hindering the development of novel treatments. This study was aimed at characterizing the transcriptional profile of successful vs. unsuccessful response to KET in mice that failed to respond to an initial course of FLX as a model of treatment resistance. We exposed adult male mice to chronic social defeat stress (CSDS), a validated mouse model for the study of depression that differentiates between resilient and susceptible mouse populations based on the social interaction test (SIT), which is highly correlated with numerous other behavioral outcomes. Mice exhibiting reduced social interaction were classified as susceptible and underwent antidepressant treatment with FLX (160 mg/L/day) in their drinking water for 28 days. A group of susceptible mice received water during the same period (water-treated). After FLX treatment, we identified a subset of mice (~35%) that continued to show reduced social interaction despite treatment (non-responders). FLX non-responders and water-treated mice were subsequently given a single injection of KET (10mg/kg IP) and behavior assessed in the SIT 24 h later. Transcriptome-wide changes in the prefrontal cortex (PFC) and nucleus accumbens (NAc) 48 h after KET administration were profiled by RNA-sequencing. We found that ~50% of FLX-non-responder mice exhibited an antidepressant response to a single KET injection, a significantly greater response than that seen in susceptible mice treated with water (0%), suggesting that FLX primes mice for successful antidepressant response to KET. We further identified a subset of treatment resistant mice who failed to respond to consecutive FLX and KET treatment. Pattern analysis of the differentially expressed genes in the PFC and NAc revealed transcriptional profiles associated with the antidepressant-like actions of FLX and of KET as well as a series of genes that were unique to treatment resistance to both drugs. We developed a novel paradigm of treatment resistance in mice that allows for the molecular interrogation of potential mechanisms that underlie antidepressant treatment resistance. The KET response rate in FLX-non-responders is similar to that seen in TRD patients, lending further validity to our model. Moreover, our findings suggest that prior unsuccessful antidepressant treatment induces a “priming effect” that increases the likelihood of successful response to KET. We are now performing weighted gene co-expression network analysis (WGCNA) to identify novel “key driver genes” that may mediate treatment resistance and response across the PFC and NAc, as the encoded proteins could represent targets for the development of novel therapeutics for TRD.

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## Blood MicroRNAs As A Biomarker For Stress Susceptibility or Resilience and Treatment Response in Major Depressive Disorder

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Major depressive disorder (MDD) is an episodic form of mental illness that is characterized by mood disturbances, anhedonia, and alterations in cognitive function. There is an urgent need for objective biomarkers for diagnosing depression, assigning treatment, and assessment of treatment response. MicroRNAs (miRNAs) are small noncoding RNA molecules, which can be detected in body fluids and have emerged as potential biomarkers of disease conditions, including depression. These molecules act as potent epigenetic post-transcriptional regulators of gene expression. Interestingly, miRNAs can be detected in the circulation and evidence suggests correlation between specific circulating miRNAs levels and several disease states. The present study explored the potential use of miRNAs as biomarkers for MDD and for prediction and assessment of treatment response. We profiled the expression levels of approximately 600 circulating blood miRNAs from mice that was collected before and after exposure to chronic social defeat stress (CSDS), as well as after either repeated imipramine or single-dose ketamine treatment. We observed robust differences in blood miRNA signatures between resilient and susceptible mice after an incubation period but not immediately after exposure to stress. Furthermore, treatment with ketamine, but not imipramine, re-established baseline miRNA expression levels in mice that responded to the drug, but not in non-responders. Analysis of candidate miRNAs in human blood samples validated a subset identified in mice as candidate biomarkers to aid depression diagnosis and predict ketamine treatment response. Lastly, we demonstrate that systemic manipulation of one of these validated miRNA targets is sufficient to reduce the depression-related phenotype in susceptible mice after stress, without an appreciable effect in resilient or control mice. Taken together, this study enhances our understanding of epigenetic changes in response to stress and identifies candidate miRNAs that warrant further investigation as biomarkers for depression treatment response.

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## The Role of $\Delta$ FosB in the Development of Drug Addiction: Identifying $\Delta$ FosB Transcriptional Targets in Nucleus Accumbens

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Drug addiction exacts devastating impact on drug users and a tremendous burden on their family members, the economy, and the nation's public health. Although different in chemical structures and initial mechanisms affecting the brain, all classes of drugs of abuse induce the expression of the transcription factor  $\Delta$ FosB in nucleus accumbens (NAc), the central node of the reward circuit. Genetically overexpressing  $\Delta$ FosB in mouse NAc neurons increases cocaine-elicited compulsive behaviors, suggesting that the expression of  $\Delta$ FosB in NAc is involved in the development of addiction. However, the molecular mechanisms of  $\Delta$ FosB remain incompletely understood. Here, we extend early work on revealing  $\Delta$ FosB transcriptional targets with chromatin immunoprecipitation (ChIP)-chip methodology, which interrogated promoter regions only, by leveraging the newer ChIP method, CUT&RUN (cleavage under targets and release using nuclease), to locate genomic loci with  $\Delta$ FosB coverage genome-wide upon chronic cocaine exposure. Male and female mice ~8 to 10 weeks of age underwent 7 days of intraperitoneal cocaine or saline injections, and bilateral punches of NAc were collected 24 hours after the last administration. Nuclei were isolated from NAc tissue with discontinuous sucrose gradients and subjected to CUT&RUN procedures. Thousands of  $\Delta$ FosB binding sites were revealed through this approach, and, interestingly, one third of the loci are positioned at distal intergenic regions while less than 10 percent of the peaks are placed within 1 kilobase from known transcription start sites, suggesting that a primary function of  $\Delta$ FosB is coordinating distal regulatory elements with transcription machinery. NAc is composed mainly of medium spiny neurons (MSNs) expressing either dopamine receptor D1 or D2;  $\Delta$ FosB is prominently induced in D1-type MSNs after most drugs (only opioids induce  $\Delta$ FosB in both cell types), and directs differential synaptic plasticity in these two types of neurons. Further extension of this CUT&RUN approach to D1- and D2-type MSNs within NAc will set the groundwork for understanding distinct roles of MSNs, and their adaptations to chronic drug exposure, in drug addiction.

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## Proteomic Profiling Of Nucleus Accumbens And Prefrontal Cortex Synaptosomes Following Withdrawal From Cocaine And Heroin Self-administration.

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Addiction is a devastating disorder that is exceptionally difficult to treat due to the high propensity for relapse even long after terminating use. The persistence of addiction is mediated at least in part by drug-induced changes in the physiology of reward-processing regions of the brain. Dysregulated signaling within the nucleus accumbens (NAc) and prefrontal cortex (PFC) are thought to play a critical role in promoting drug-seeking and relapse. Determining these changes may reveal more effective targets to treat substance use disorders. However, the molecular details underlying these adaptations remain incompletely understood. Here, we extend previous work focused mainly on candidate proteins of interest by contrasting whole-proteome changes induced by cocaine or heroin self-administration that persist through extended abstinence. Rats underwent 10 days of extended-access (6h) intravenous cocaine, heroin, or saline self-administration followed by 30 days of forced abstinence, after which rats were euthanized from the homecage and the NAc and PFC were extracted. Synaptosomes from these regions were purified and run on lipid chromatograph tandem (LC-MS/MS) mass spectrometry followed by label-free quantification. Using this approach, we have identified unique patterns of induction or repression of numerous synaptic proteins in NAc and PFC following prolonged withdrawal from cocaine or heroin self-administration. Interestingly, long-term withdrawal is associated with a greater degree of repression rather than induction of protein expression overall. Further, the NAc exhibits a greater degree of unique synaptic protein changes following withdrawal from cocaine, while the PFC is most affected by withdrawal from heroin. We are now validating these findings and characterizing particular synaptic proteins that directly contribute to drug-seeking behavior. These studies will dramatically enhance our understanding of the molecular restructuring of synapses throughout the reward circuitry that underlie susceptibility to relapse.

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