Neuroepigenetics of Cocaine Use:

A Positron Emission Tomography Study of Histone Deacetylase Expression in People Who Use Cocaine

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ABSTRACT

With repeated use, cocaine can induce long-lasting changes in the brain's reward circuitry that increase susceptibility to compulsive drug seeking and taking. Studies in laboratory animals raise the possibility that the molecular mechanisms fostering these changes include epigenetic processes that alter gene expression patterns. The present research measured striatal expression of the epigenetic enzymes, histone deacetylases (HDACs), in cocaine users, using positron emission tomography (PET) with [^{11}C]Martinostat, the first ever validated method for measuring HDAC expression in living human brain. The main results suggest that frequency of recent cocaine use positively predicts HDAC expression in ventral and dorsal parts of the striatum, whereas time since last cocaine use negatively predicts HDAC expression in the ventral striatum. Together, these results suggest that cocaine use leads to transient increases in striatal HDAC expression that may be maintained with frequent use. HDAC expression in the associative striatum was also positively correlated with early life adversity but a mediation effect was not observed, raising the possibility that early life stress may have lasting effects on striatal HDAC expression distinct from cocaine use. These findings provide first ever insight into cocaine-induced changes in HDAC expression in the human brain and opens the field to questions investigating cocaine use severity and time-specific effects. This line of research has the potential to characterize links between environmental events and epigenetic processes, elucidating molecular pathways to addiction.

RÉSUMÉ

La consommation répétée de cocaïne peut induire des changements de longue durée dans les circuits de récompense du cerveau qui augmentent la susceptibilité aux comportements liés à la toxicomanie. Les études chez les animaux de laboratoire suggèrent que les mécanismes moléculaires favorisant ces changements comprennent des processus épigénétiques qui modifient les modèles d'expression génétique. La présente étude a mesuré l'expression striatale des enzymes épigénétiques, histone désacétylases (HDAC), chez les consommateurs de cocaïne, au moyen de la tomographie par émission de positrons avec [^{11}C]Martinostat, la première méthode validée pour quantifier l'expression des HDAC dans le cerveau humain vivant. Les principaux résultats suggèrent que la fréquence de consommation récente de cocaïne prédit de façon positive l'expression des HDAC dans le striatum ventral et dorsal, tandis que le temps écoulé depuis la dernière utilisation de cocaïne prédit de façon négative l'expression des HDAC dans le striatum ventral, impliquant que la cocaïne peut entraîner des augmentations transitoires de l'expression striatale des HDAC qui peuvent être maintenues par la consommation fréquente. L'expression des HDAC dans le striatum associatif a aussi été positivement corrélée avec l'adversité au début de la vie, mais des effets de médiation n'ont pas été observés, ce qui suggère que le stress au début de la vie peut avoir des conséquences durables sur l'expression striatale des HDAC, distinctes de celles de la cocaïne. Ces résultats fournissent un premier aperçu des changements de l'expression des HDAC induits par la cocaïne dans le cerveau humain, et soulèvent des questions portant sur la sévérité de la consommation de cocaïne et les effets propres au temps. Cet axe de recherche a le potentiel de définir les liens entre des expositions environnementales et des processus épigénétiques, et d'éclaircir les voies moléculaires menant à la toxicomanie.

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CONTRIBUTION OF AUTHORS

CHAPTER 1: INTRODUCTION

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CHAPTER 2: METHODOLOGY

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CHAPTER 3: RESEARCH FINDINGS

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CHAPTER 4: DISCUSSION

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CHAPTER 1: INTRODUCTION

Drug addiction is a leading cause of disability in the world today. In Canada alone, the non-medical use of drugs is associated with an estimated economic cost of $46 billion per year (Canadian Substance Use Costs and Harms Scientific Working Group, 2020). In the United States, a large portion of drug overdose deaths in the past decade are attributable to the use of opioids; however, deaths linked to cocaine use have been on the rise (Warner et al., 2016), with cocaine related deaths (including crack cocaine) among non-Hispanic black men and women surpassing those from opiate overdose between 2000 and 2015 (Shiels et al., 2018). Moreover, young adults have the highest percentage of current cocaine users with an estimated 29,000 adolescents in the United States having a cocaine use disorder (Ryan, 2019).

Our understanding of the biological mechanisms that initiate and maintain maladaptive cocaine use patterns remains incomplete. Some progress has been made, though, and drug use is thought to induce neuroplastic changes that lead to compulsive drug seeking and taking despite adverse consequences. The resulting addictive phenotype is enduring, increasing susceptibility to drug use (Berridge, 2017; Stewart 2008) and clinical relapse (Sinha, 2011) even following extended periods of abstinence.

Strikingly, however, only a subset of individuals using addictive substances progresses to a substance use disorder. Twin and adoption studies suggest that this differential vulnerability reflects complex interactions between inherited genes and environmental events, such as exposure to chronic stress and drug use itself (Bierut et al., 1998; Kendler et al., 2012). One compelling candidate mechanism that might mediate this gene-environment interaction is the family of epigenetic processes. These processes operate throughout life, regulating the expression of certain

genes and priming others for induction or repression in response to drugs and other events (Walker et al., 2015).

Most addiction related epigenetic research has been in laboratory animals, and little is known about epigenetic processes in living human brain. To bridge this gap between laboratory and human conditions, the present study measured the expression of the epigenetic enzymes, histone deacetylases (HDACs), in people who use cocaine, using the recently developed positron emission tomography tracer, [^{11}C]Martinostat.

1.1. Neurobiology of Cocaine Addiction

Most drugs of abuse, including cocaine, exert their initial reinforcing effects on the brain's mesocorticolimbic circuitry. These pathways regulate responses to rewards and punishments, attribute motivational salience to events, and promote situation-appropriate planning to either approach or avoid (Leyton & Vezina, 2014; Lüscher, 2016) enabling us to navigate our way away from potential dangers and toward natural rewards, like food, sex, and social interaction. Drugs of abuse can redirect these motivational processes through persistent and stable changes in neuronal structure and function and may cause an individual to lose control over their drug consumption patterns despite the negative consequences (Nestler, 2005).

1.1.1. Role of Dopaminergic Signalling

Mesolimbic dopaminergic neurons play a key role in reward-seeking behaviours. These neurons originate in the midbrain ventral tegmental area (VTA) and release dopamine in diverse terminal regions including the nucleus accumbens (NAc) and other areas within the ventral

striatum, other more dorsal aspects of the striatum, the prefrontal cortex, parts of the thalamus, and the amygdala (Lüscher & Ungless, 2006). Electrical activation of NAc afferents (Gratton et al., 1988; Roberts et al 1977) and selective optogenetic excitation of VTA dopamine neurons (Pascoli et al., 2015) can heighten the ability of motivationally relevant stimuli to elicit and sustain approach and increase the probability that a behaviour will be repeated.

For cocaine, a critical step in the precipitating neurochemistry occurs when the drug binds to presynaptic dopamine reuptake transporters (DAT) (Kuhar et al., 1988). By blocking them, cocaine leads to elevated extracellular dopamine concentrations. This DAT blockade in the ventral striatum appears to be the primary mediator of cocaine's rewarding and reinforcing effects (Nestler, 2005), as both systemic and direct intra-cranial injections of dopamine receptor antagonists disrupt cocaine reinforcement in laboratory animals (de Wit & Wise, 1977; Maldonado et al., 1993; McGregor & Roberts, 1993).

With repeated use, cocaine can lead to long-lasting changes to dopamine transmission and dopamine-related behaviours. Both in humans and rodents, multiple exposures to moderate to high doses of cocaine can induce sensitization, marked by progressive increases in drug-induced behavioural activation, greater willingness to sustain effort to obtain drug reward, and greater drug-induced dopamine release (Boileau et al 2006; Leyton & Vezina, 2014). Through conditioning, environmental cues paired with the drug can come to also induce behavioural activation and dopamine release similar to cocaine itself (Boileau et al 2007; Stewart et al., 1984; Wong et al., 2006). This combination of drug-induced sensitization, conditioning, and individual differences in susceptibility to these effects (Leyton & Vezina, 2014) can drive progressively more frequent drug use, paving the way to a substance use disorder.

1.1.2. Striatal Circuity, Habits, and Compulsion

While cocaine cue-induced dopamine responses are clearly demonstrated in the ventral striatum during initiation of drug use (Boileau et al 2007; Ito et al 2000), following repeated exposure to cocaine and the development of habitual behaviour, the largest responses are observed in the dorsal striatum (Cox et al 2017; Ito et al., 2000, 2002). This shift of conditioned dopamine responses from the ventral to the dorsal striatum is thought to promote the formation of a second aggravating feature commonly seen in addictions, the development of habits.

Habit formation is characterized by a transition from instrumental behaviours under the control of the goal-directed system, where action performance will be reduced following outcome-specific devaluation (Adams & Dickinson, 1981; Dickinson, 1985), to habitual responses reliant on a stimulus-response associative architecture (Dickinson, 1985; Everitt & Robbins, 2016). Repeated cocaine exposure regimens in laboratory animals can promote habit formation that results in cocaine-seeking behaviours that are insensitive to negative consequences (Leblanc et al., 2013; Zapata et al., 2010).

The goal-directed and habit systems are preferentially mediated by the dorsomedial (DMS) and dorsolateral (DLS) striatum respectively (Murray et al., 2012; Yin et al., 2004, 2005). In humans, these regions approximately correspond to the associative striatum, which includes dorsal aspects of the caudate nucleus and anterior dorsal putamen, and the sensorimotor striatum, which mainly constitutes the posterior putamen (Parent & Hazrati, 1995). In rats extensively trained in cocaine self-administration, a unilateral lesion disconnecting NAc and DLS can decrease cocaine-seeking behaviours (Belin & Everitt, 2008), suggesting that interactions between ventral and dorsal striatum may be critical in the development of addiction. Since compulsive drug use may be

aggravated by an inability to disengage from these dorsal striatum-regulated habits (Giuliano et al., 2019), neuroadaptations in both the ventral and dorsal striatum are of interest.

1.1.3. Early Life Adversity as a Risk Factor

The effects of acute and repeated cocaine use on striatal dopamine transmission may account in large part for the persistent potentiation of drug-related behaviours, but it remains poorly understood why these processes lead to compulsive drug use only in a subset of individuals. Risk factors thought to contribute to differential vulnerability to addiction include heritable, genetic factors, with consistent evidence for heritability in twin studies (Goldman et al., 2005). However, the initiation of substance use and the transition to clinically problematic use has also been associated with environmental factors, including peer pressure, unhealthy family dynamics, and early life adversity (Kendler et al., 2012).

In humans, adversity experienced during childhood and adolescence, such as emotional, physical, and sexual abuse, and emotional and physical neglect, is associated with a markedly increased risk of later substance use disorders (Teixeira et al., 2017; Van Dam et al., 2014) and other mental health conditions, such as mood and anxiety disorders (Espejo et al., 2007; Heim et al., 2008; McLaughlin et al., 2020; Merrick et al., 2017). In rodents, the effects of early life adversity have been studied in various paradigms. Prepubescent social isolation in rodents results in a long-lasting increase in the motivation to self-administer cocaine (Baarendse et al., 2014; Fosnocht et al., 2018) and repeated maternal separation in postnatal mice leads to heightened sensitivity to the effects of cocaine during adulthood (Kikusui et al., 2005). Similarly, social defeat stress, consisting of an antagonistic encounter between a dominant and subordinate animal, during

adolescence is associated with increased novelty seeking and risk taking (Watt et al., 2009) and increases in conditioned place preference for cocaine (Lo Iacono et al., 2016).

Stressful events can also potentiate dopamine release in the ventral striatum, via increased levels of glucocorticoids (Brake et al., 2004; Dallman et al., 1994; Doherty & Gratton, 1992; Meaney et al., 2002; Sorg & Kalivas, 1991), and enhance responses to subsequent stressors in a fashion similar to drug-induced sensitization (Kalivas & Stewart, 1991), hinting at a shared ability to alter structure and function in the mesolimbic brain regions and cause corresponding changes in reward processing. This view is substantiated by the observation of cross-sensitization between stress and drugs of abuse, where repeated stress exposure is shown to increase behavioural and dopamine responses to drugs of abuse (Antelman et al., 1980; Booij et al., 2016; Kalivas & Stewart, 1991; Leyton & Stewart, 1990; Matuszewich et al., 2014; Meaney et al., 2002). This convergence of effects between stress and cocaine on reward-related behaviour and dopamine responses underlines the potential role for a shared biological mechanism that can explain both the persistence of addiction pathophysiology and the life-long susceptibility to developing an addictive phenotype resulting from early life adversity.

1.1.4. Changes in Gene Expression and Cocaine-Evoked Synaptic Plasticity

The effects of both repeated cocaine use and early life adversity on reward processing include stable molecular adaptations in the brain (Madsen et al., 2012). Since many forms of these effects are dependent on protein synthesis, numerous studies have focused on the regulation of gene expression as a mechanism through which these adaptations can be induced, in a transcription-dependent manner. A large number of transcription factors, including *Fos*, *FosB*, *Nr4a2*, and *CREB*, and an even greater number of proteins regulated by them, like *BDNF* and *Cdk5*,

can be upregulated in the ventral and dorsal striatum following acute and repeated cocaine exposure (Mews & Calipari, 2017; Nestler, 2005). The signaling pathways downstream of these molecules are pivotal for maintaining cellular memory and strengthening and weakening synaptic connections required for persistent changes in behaviour (Lüscher, 2016).

Stress engages similar neuroplastic processes. For example, following chronic emotional or physical stress, expression of *Fos*, *FosB* and *BDNF* can increase in the striatum (Flak et al., 2015; Krishnan et al., 2007), once again highlighting the commonality of effects between stress and drugs. Animal studies investigating effects of early life adversity on circuit development and neural responses to stress and reward in adulthood suggest that these transcriptional changes, driven by early life adversity, are sustained across the lifespan (Peña et al., 2017, 2019).

Transcription factors constitute a key link between extrinsic stimuli, like cocaine or stress, to intrinsic responses, like changes in gene expression. However, transcriptional regulation involves, beyond the activity of transcription factors themselves, alterations to the transcriptional potential of neurons at the chromatin level making DNA more or less accessible to the transcriptional machinery. The most compelling candidate mechanisms behind these alterations are epigenetic.

1.2. Epigenetics of Cocaine Use

DNA is compactly condensed in the cell nucleus within a complex called chromatin, where it is wrapped around octamers of histone proteins. Following the composition of a DNA molecule, various modifications can occur to the histones, including acetylation, methylation, and phosphorylation. These ‘post-translational’ modifications influence the “readability” of the DNA

double helix, altering the likelihood of gene transcription at a given locus. These and other modifications to gene expression that do not arise from changes in DNA sequence are called 'epigenetic' (Bard, 2008). Together, the various epigenetic changes sculpt the structure and functional state of regulatory elements in the genome.

Addictive drugs can influence epigenetic mechanisms both directly, by activating its molecular target and downstream signalling cascades, and indirectly, through increased dopamine signalling and its downstream cascades (Nestler & Lüscher, 2019). Considering the diversity of molecular targets for different classes of drugs, the indirect influence appears to be particularly important for the development and maintenance of addictive phenotypes. Considering the involvement of epigenetic mechanisms in events during early development and resulting permanent molecular changes (Honegger & de Bivort, 2018), it is possible that certain epigenetic modifications contribute to the enduring pathophysiology of and differential susceptibility to addictions.

1.2.1. Histone Acetylation

Histone acetylation has been the most comprehensively studied epigenetic mechanism in the field of cocaine addiction (Nestler & Lüscher, 2019; Rogge & Wood, 2013). It involves the transfer of an acetyl group onto the positively charged lysine residue within the N-terminal tail of a histone protein, negating the positive charge, and thus inducing an "open" chromatin state, permitting greater transcription factor binding (Pokholok et al., 2005).

Acute and repeated exposures to cocaine have been linked to increased acetylation levels at histones H3 and H4 in the striatum (Kumar et al., 2005; Levine et al., 2005, 2011; Renthal et al., 2009). Since both H3 and H4 acetylation are markers of transcriptional activation (Kouzarides,

2007), cocaine's predominant effect appears to be activating gene transcription. However, as shown by chromatin immunoprecipitation assays in rats, cocaine exposure does not enhance H3 and H4 histone acetylation in a global, genome-wide manner, but at the promotors of genes previously implicated in cocaine-induced neuroplasticity, such as *Fos, FosB*, *Bdnf II*, and *Cdk5* (Kumar et al., 2005; McClung & Nestler, 2003).

Cocaine-mediated changes in histone acetylation have been mapped genome-wide in the rodent ventral striatum (Renthal et al., 2009; Sun et al., 2017). Different cocaine administration regimens lead to different effects on promoter acetylation. Namely, acute cocaine administration increases H4 acetylation at the *Fos* and *FosB* promoters, with no effect on *Bdnf II* or *Cdk5* promoters, whereas a repeated regimen, including self-administration, elevates H3 acetylation at *FosB*, *Bdnf II*, and *Cdk5* promoters, but not at the *Fos* promoter (Kumar et al., 2005; Wang et al., 2010). These findings are consistent with cocaine's ability to induce *Fos* family transcription factor expression acutely and *Bdnf II* or *Cdk5* expression only after more extensive exposure (Daunais et al., 1993; Hope et al., 1992; McClung & Nestler, 2003).

Given the specificity of H3 and H4 acetylation profiles, cocaine's regulation of histone acetylation most likely occurs selectively via specific histone modifying enzymes, such as histone acetyltransferases (HATs) and histone deacetylases (HDACs). Consequently, cocaine-mediated changes in histone acetylation levels may reflect the net result of alterations to the balance of HAT and HDAC function.

1.2.2. Effects of Cocaine on Histone Deacetylase Function

HDACs remove acetyl groups from lysine residues and tighten the chromatin structure, diminishing DNA availability to transcription factors and decreasing gene expression. Following

exposure to stimulant drugs, HDACs are recruited to promoter regions of certain genes in the striatum (Kennedy et al., 2013; Rogge et al., 2013). Studies in rodents manipulating HDAC activity have revealed a link between HDAC-mediated changes in histone acetylation and cocaine-induced behavioural responses (Walker et al., 2015); specifically, pharmacological HDAC inhibitors, such as sodium butyrate, valproate, and trichostatin A, were shown to modulate both transcriptional and behavioural responses to cocaine. Given that these inhibitors selectively block the activity of class I HDACs, namely HDAC1, 2, 3, and 8 (Kilgore et al., 2010), these findings suggest that class I HDACs may be of particular interest in their role regulating neural responses to cocaine.

Nonetheless, studies on the effects of general class I HDAC inhibition on cocaine-related behaviours have yielded conflicting results. Some studies report that a systemic or ventral striatal inhibition of class I HDACs leads to increased locomotor sensitization and self-administration (Kumar et al., 2005; Sun et al., 2008; Wang et al., 2010; Itzhak et al., 2013), while other studies report that it facilitates extinction to cocaine-related cues and decreases relapse behaviours as well as cocaine self-administration both when it is easy and difficult to obtain (Malvaez et al., 2010; Romieu et al., 2008, 2011).

A comparison of these studies reveals that an acute inhibition of HDACs enhances behavioural effects of cocaine, whereas chronic regimens attenuate them (Kennedy et al., 2013). Concordant with this, cocaine-induced behavioural and transcriptional plasticity was blunted by a prolonged and selective knockdown of HDAC1 and HDAC2 in the NAc (Kennedy et al., 2013) and HDAC3 (Hitchcock et al., 2019; Malvaez et al., 2013). Interestingly, a focal deletion of NAc HDAC3 prior to cocaine exposure enhanced cocaine-induced conditioned place preference (Rogge et al., 2013), suggesting that the behavioural outcomes of reduced HDAC activity are dependent on not only the length but also the precise timing of the inhibition. Both HDAC inhibitors and

cocaine are associated with increased histone acetylation and thus permissive transcription states (Kumar et al., 2005; Schroeder et al., 2013). When co-administered acutely, they may have synergistic effects, inducing increased gene expression and behavioural sensitization, whereas when cocaine is administered following sustained, HDAC inhibitor-induced hyperacetylation, homeostatic processes may take place, diminishing the already enhanced transcriptional activation (Kennedy et al., 2013).

The picture becomes more complicated when interactions between different classes of HDACs are taken into consideration. Selective knockdown of HDAC1 causes a compensatory upregulation of Class IIa HDAC, HDAC5, and downregulation of Class III HDAC and Sirtuin 1 (SIRT1) expression. Following repeated cocaine exposure, HDAC5 is downregulated in the NAc and its inhibition has been linked with cocaine hypersensitivity (Renthal et al., 2007), whereas SIRT1 is upregulated in the NAc and its inhibition attenuates behavioural responses to cocaine (Renthal et al., 2009). Further studies are required to determine to what extent this compensatory regulation of HDAC5 and SIRT1 contributes to the behavioural and molecular consequences of changes in Class I HDAC expression.

Although cocaine is linked with increased histone acetylation, quantitative PCR and immunohistochemistry studies revealed that repeated cocaine self-administration is followed by an increase in HDAC2 mRNA expression in the rat ventral and dorsal striatum (Cassel et al., 2006; Host et al., 2011). In mice, this increase in HDAC2 mRNA levels is not observed in the ventral striatum after 24 hours since last cocaine intake (Renthal et al., 2007), suggesting that HDAC2 expression in response to cocaine may be regulated in a transient manner. Of interest, a similar increase in HDAC2 expression in the ventral striatum is also demonstrated following an acute injection of another central nervous system stimulant, methamphetamine (Martin et al., 2012).

These seemingly paradoxical findings imply the existence of complex and potentially compensatory interactions between class I HDACs and other epigenetic enzymes.

Overall, while the relationship between class I HDAC activity and cocaine-induced acetylation is not straightforward, studies in laboratory animals suggest that repeated exposure to stimulant drugs transiently increases HDAC2 expression in the striatum whereas sustained inhibition of striatal class I HDACs is associated with a mitigation of cocaine's enduring behavioural effects.

1.2.3. Effects of Stress on Histone Deacetylase Function

A smaller number of studies have investigated HDAC-related mechanisms in the maintenance of persistent behavioural changes driven by both acute and chronic stress (Bagot et al., 2014). The first demonstration of such a role was in laboratory animals when pharmacological HDAC inhibition attenuated stress-induced depression and anxiety-like behaviours (Covington et al., 2009; Moloney et al., 2015; Tsankova et al., 2006; Uchida et al., 2011) and molecular changes involving gene expression (Ieraci et al., 2015; Wang et al., 2017).

Similar to cocaine (Cassel et al., 2006; Host et al., 2011), early life adversity, in the form of maternal separation, has been shown to induce higher levels of HDAC2 mRNA, coupled with decreased H3 and H4 acetylation, in the ventral and dorsal striatum (Tesone-Coelho et al., 2013). However, chronic social defeat stress experienced during adulthood had resulted in decreased HDAC2 expression (Covington et al., 2009), suggesting differential molecular consequences of stressors across the lifespan. Similarly, in the rodent forebrain, maternal separation and maltreatment were shown to decrease expression of HDAC1 and HDAC3 (Blaze & Roth, 2013;

Levine et al., 2012), indicating brain region and isoform-specific effects of stress on transcription of HDACs.

Taken together, the background literature in animal models suggests that while repeated cocaine use induces increased class I HDAC expression and activity in the striatum, potentially driving sensitized and conditioned responses to subsequent cocaine use, changes in HDAC expression may also occur prior to drug use, as a result of early life exposure to adverse events, functioning essentially as a mediator between early life adversity and drug use outcomes.

1.3. Imaging Epigenetic Processes in the Human Brain

Localized dysfunction of HDACs have been demonstrated in the context of various psychiatric conditions beyond substance use disorders (Volmar & Wahlestedt, 2015); however, most research on this topic has been in laboratory animals. Consequently, a translational gap remains between laboratory and human conditions. The use of post-mortem human brain tissue in this pursuit is complicated by diagnostic challenges of identifying ante-mortem illicit substance use (Lehrmann et al., 2008) and relative instability of immunoreactivity for histone acetylation at 4-5 days post-mortem (Jafari et al., 2014; Jarmasz et al., 2019).

1.3.1. PET Imaging with [^{11}C]Martinostat

Many preclinical studies suggest that HDACs may be promising therapeutic targets in addiction. However, until recently, there had been no validated method to visualize and quantify *in vivo* epigenetic mechanisms in the human brain. This changed with the development of the PET radiotracer [^{11}C]Martinostat.

[^{11}C](E)-3-(4-((((3r,5r,7r)-Adamantan-1-ylmethyl)(methyl)amino)methyl)-phenyl)-N-hydroxyacrylamide, also known as [^{11}C]Martinostat, selectively and reversibly binds to class I HDACs (isoforms 1, 2, and 3), as well as one class IIb HDAC (isoform 6) with low nanomolar affinities (Wang et al., 2014). Since brain levels of HDAC6 are very low, the tracer's signal is dominated by HDAC1, 2, and 3 (Wey et al., 2016). Previous non-invasive probes for imaging HDAC enzyme density *in vivo* either exhibit poor brain penetrating ability (e.g. [^{11}C]MS-275, [^{11}C]BA, [^{11}C]PBA, [^{11}C]VPA, [^{18}F]SAHA) (Hendricks et al., 2011; Hooker et al., 2010; Kim et al., 2013; Seo et al., 2013) or bind to isoforms that have not been extensively implicated in disease in animal models (e.g. [^{18}F]FAHA) (Yeh et al., 2013). However, [^{11}C]Martinostat has robust uptake and high specific binding to class I HDACs in the brain, with a dose of 1 mg/kg reaching approximately 99% occupancy, and other peripheral organs (Schroeder et al., 2014; Wang et al., 2014, 2015).

The sensitivity of the radiotracer to resolve differences in HDAC expression was also shown in rodents and baboons via both self-blocking experiments using unlabeled Martinostat and blocking experiments using the class I HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA) (Wang et al., 2014). Similarly, in rats, [^{11}C]Martinostat binding was shown to decrease when co-administered with other HDAC-binding compounds, including *ortho*-amino-anilide HDAC inhibitors, CI-994, Cpd60, and RGFP966, and class I HDAC inhibitor valproate (Schroeder et al., 2014). These homologous and heterologous competition assays demonstrate a high proportion of specific binding for the radiotracer.

The kinetic properties of [^{11}C]Martinostat were characterized in baboons (Wang et al., 2015). Maximal uptake was reached within 20 minutes and the dynamic data best fit a two-tissue compartmental model. The analysis of individual rate constants estimated through kinetic

modeling revealed rapid uptake of the radiotracer from the bloodstream (large K_1 and small k_4). Moreover, k_2 was larger than k_3 by a factor of 2, suggesting that the radiotracer is likely insensitive to blood flow changes and arterial sampling is not needed.

Ex vivo biochemistry of post-mortem human and baboon brain tissues from both grey and white matter revealed that the [^{11}C]Martinostat signal in the brain originated from binding HDAC isoforms 1, 2, and 3 (Wey et al., 2016). In thermal shift assays, Martinostat did not significantly stabilize HDAC6, suggesting that radiotracer binding may primarily reflect the enzyme density of class I HDACs. To date, no significant off-target sites have been identified.

1.3.2. Histone Deacetylase Imaging in Humans

Wey and colleagues (2016) translated [^{11}C]Martinostat for clinical research use and quantified, for the first time, *in vivo* human epigenetic regulation. In the human brain, [^{11}C]Martinostat uptake showed minimal decrease during a 90-minute PET scan. Researchers noted that inter-subject variability was smaller using an analysis of standardized uptake values (SUV) than distribution volume (V_T). The consistency of [^{11}C]Martinostat binding patterns between individual subjects observed in this study highlights the importance of region-specific levels of HDACs in brain function and hints at the detectability of deviations from these patterns as potential pathological biomarkers in HDAC dysfunction-associated populations.

Considering the risks and invasiveness of arterial blood sampling, [^{11}C]Martinostat standard uptake value ratios (SUVR) has been suggested as a good parameter for ligand quantification. Donovan and colleagues (2020) demonstrated a strong correlation between *in vivo* SUVR and *in vitro* measures of HDAC1, 2, and 3 protein expression in the pig brain, supporting that [^{11}C]Martinostat SUVR provides a good measure of cerebral HDAC protein levels. Because

HDACs are expressed globally in the brain, an ideal reference region cannot be identified. In pigs, the olfactory bulbs were selected as a pseudo-reference as the region with the lowest radiotracer uptake. However, in humans, it is shown that using the whole brain mean as a pseudo-reference region is an appropriate surrogate measure for V_T (Gilbert, Zürcher, Catanese, et al., 2019; Gilbert, Zürcher, Wu, et al., 2019; Tseng et al., 2020), simultaneously allowing for removal of inter-individual differences in global signal.

These recent studies have used PET with [^{11}C]Martinostat to investigate HDAC expression and distribution in people with schizophrenia (Gilbert, Zürcher, Wu, et al., 2019) and bipolar disorder (Tseng et al., 2020). Moreover, it was demonstrated that HDAC expression increases with age in the cerebral white matter, and that [^{11}C]Martinostat SUVR was higher in females than males in the hippocampus, amygdala, and parts of the thalamus, but not in the striatum (Gilbert, Zürcher, Catanese, et al., 2019). However, to date, no study has demonstrated, in living human brain, effects of psychoactive drug use or early life adversity on HDAC distribution or expression.

1.3.2. Histone Deacetylase Imaging in People Who Use Cocaine

The current study used PET with [^{11}C]Martinostat to measure the expression of HDAC1, 2, and 3 in cocaine users and stimulant-drug naïve healthy controls, constituting, to our knowledge, the first investigation of *in vivo* HDAC expression in the human brain in a population of substance users.

In laboratory animals, cocaine-induced changes in striatal HDAC expression appear to induce changes in gene expression patterns that lead to potentiated behavioural responses to subsequent cocaine use. In addition, an established risk factor for drug addiction, early life adversity appears to engage similar neuroplastic processes in dopaminergic striatal pathways,

raising the possibility that striatal HDAC expression may act as a mediator mechanism. In light of this, the current study aims to (1) measure the effects of cocaine use on HDAC expression in the ventral and dorsal striatum, as measured by [^{11}C]Martinostat SUVR with whole brain as the reference, (2) investigate the relationship between striatal HDAC expression and cocaine-use variables, such as lifetime use, current frequency use, and time since last use, and (3) quantify the association between childhood trauma and striatal HDAC expression, including whether the effect of childhood trauma on cocaine-use outcomes are mediated by striatal HDAC expression.

Based on the findings in laboratory animals reviewed above, it is predicted that (1) striatal HDAC expression will be higher in subjects intermittently using cocaine compared to stimulant-naïve controls, (2) among subjects who use cocaine, striatal HDAC expression will positively correlate with lifetime occurrences and current frequency of cocaine use and negatively correlate with time of abstinence since last use, and (3) striatal HDAC expression will positively correlate with the measure of childhood trauma and mediate its positive effect on cocaine use status.

CHAPTER 2: METHODOLOGY

2.1. Study Design

The primary research objective was to measure *in vivo*, striatal expression of HDAC1, 2, and 3 in stimulant drug-naïve controls and cocaine users and investigate its association with variables of cocaine use, such as cocaine use status, frequency of cocaine use, and time since last use, as well as with early life adversity. The region-specific HDAC expression was measured using PET with [^{11}C]Martinostat, guided by anatomical magnetic resonance imaging (MRI). The primary outcome measure was the mean SUV for the striatal regions, collected between 60 and 90 minutes after radiotracer injection, normalized to the whole brain mean (SUVR).

The study was approved by McGill University Health Centre Research Ethics Board (REB) and Health Canada. All participants provided written consent in accordance with the Declaration of Helsinki, underwent a physical examination, and completed one anatomical MRI and one PET scan at the McConnell Brain Imaging Centre at the Montreal Neurological Institute and Hospital.

2.2. Participants

2.2.1. Recruitment

Participants were recruited through advertisements placed on classified advertising websites, social media platforms, and university campuses. Volunteers were initially screened through a telephone interview and were excluded if they had a history of psychotropic medication use or hormone treatments, were younger than 18 or older than 40 years of age, had received a radiation dose exceeding 50mSv in the past 12 months, had a history of major head trauma, were pregnant or

breastfeeding, had ferromagnetic foreign bodies, or had neurological or other disorders that might be aggravated by participation in the study or complicate interpretation of the results.

From over 200 telephone screening interviews, 48 eligible volunteers were identified and invited to sign a consent form, understanding of which was ensured using the UCSD Brief Assessment of Capacity to Consent (UBACC) (Jeste et al., 2007). Forty-three consenting volunteers were then interviewed with the Structured Clinical Interview for DSM-5 (SCID-5) to identify psychiatric symptoms and substance use severity (Shankman et al., 2018). Based on this assessment, participants were recruited into two experimental groups: (1) stimulant drug-naïve healthy controls, and (2) drug users who had used cocaine on at least six occasions in the past six months and on more than ten occasions in their lifetime. Control group volunteers were excluded if they met criteria for a current or past DSM-5 psychiatric or substance use disorder (with the exception of tobacco use disorder), while volunteers with a history of stimulant drug use were excluded if they met criteria for a current psychiatric disorder or a substance use disorder not concurrent with a cocaine use disorder. To ensure the absence of physical illness or problems likely to interfere with the assessment of brain function, candidates underwent a physical examination by a licensed physician. After this evaluation, 28 participants were deemed eligible; however, with 6 subjects withdrawing before undergoing the PET scan, a total of 22 participants (14 healthy controls and 8 cocaine users) completed the study.

2.2.2. Measures of Early Life Adversity, Mood, and Personality

During the clinical interview, participants completed a series of questionnaires to determine several behavioural measures. Information regarding adverse early life experiences was collected from each participant using the Childhood Trauma Questionnaire (CTQ) (Bernstein & Fink, 1998).

The CTQ is a 28-item measure used to provide a retrospective measure of childhood trauma in five subscales: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. It has been validated across diverse healthy and disease-impacted populations (Bernstein et al., 2003; Spinhoven et al., 2014). For the purposes of this study, the total CTQ score was selected as the predictor variable.

Other measures were also assessed as potential correlates. Personality traits, such as hopelessness, anxiety sensitivity, impulsivity, and sensation seeking, were assessed using the Substance Use Risk Profile Scale (SURPS) (Woicik et al., 2009) and the Barratt Impulsiveness Scale (BIS-11) (Patton et al., 1995). Measures of mood related to depression and anxiety were assessed with the Beck Depression Inventory (BDI) (Beck et al., 1996) and State-Trait Anxiety Inventory (STAI) (Spielberger, 1989) respectively.

2.2.3. Substance Use

Frequency of substance use was measured using a Timeline Follow-Back Method (Sobell et al., 1996) and overall lifetime use, number of occasions per month, and units per occasion were recorded for each substance a participant had used. For alcohol, information on lifetime occasions of binge drinking (more than five drinks per occasion for males and more than four for females) was also recorded. Alcohol related problems were assessed using the Alcohol Use Disorder Identification Test (AUDIT) (Saunders et al., 1993).

Participants were asked to abstain from caffeine and nicotine for at least 5 hours, alcohol for 24 hours, stimulants for 4 days, and cannabis for 7 days prior to their PET and MRI scans. A breathalyser test ensured alcohol abstinence (BACtrack S80, KHN Solutions LLC, CA, USA), whereas a urine toxicology test (VeriCheck Drug Test Cup, Verify Diagnostics, ON, Canada) was

used to screen for amphetamine, benzodiazepines, buprenorphine, cocaine, 3,4-methylenedioxy-methamphetamine, methamphetamine, methadone, opioids, and tetrahydrocannabinol (THC). Four participants who tested positive for THC but were not acutely intoxicated and reported no use for a minimum of seven days were included in the study, since even sporadic cannabis use can lead to long lasting positive urine tox results. A urine pregnancy test (Biostrip HCG, Innovatek Medical Inc., Delta, BC, Canada) was conducted in females before each brain imaging session.

2.3. Brain Imaging Procedure

2.3.1. PET Image Acquisition

[^{11}C]Martinostat was synthesized as previously described (Wey et al., 2016). Twenty-two participants underwent an 11-minute transmission scan followed by a bolus [^{11}C]Martinostat injection through an intravenous catheter placed in the antecubital vein of the arm and a 90-minute emission scan on a Siemens High Resolution Research Tomograph (HRRT, CTI/Siemens). No physiological changes or adverse reactions were noted with radiotracer administration. A 90-minute emission scan was preferred as it yields stable estimations for average total distribution (V_T) in all regions (Wey et al., 2016). All PET sessions started between 10:00AM and 03:00PM and no group differences were observed in scan start times (p=0.23). Participants were instructed to remain awake and still for the duration of the scan.

2.3.2. MRI Image Acquisition

Participants underwent a high-resolution, T1-weighted, three-dimensional volume acquisition for anatomical co-registration (1 mm^3 voxel size) on a 3T Siemens Magnetom Prisma

MRI scanner, using the MP-RAGE sequence, with the following acquisition parameters: repetition time = 2300ms, echo time = 2.96ms, flip angle = 9°, field of view = 256mm, and matrix = 256 × 256. This scan was used for co-registration purposes. Due to scheduling difficulties, MRI data were missing for two healthy controls, for whom the alignment of the PET data to anatomy was conducted manually (for details, see Section 2.3.3. Image Analysis). Additionally, one healthy control was excluded from the study because of poor quality of co-registration arising from anatomical abnormalities.

2.3.3. Image Analysis

Each T1-weighted MR image was pre-processed using the CIVET image processing pipeline (Ad-Dab'bagh et al., 2006). The native MR volumes were corrected for image intensity non-uniformity (Sled et al., 1998) and transformed into the Montreal Neurological Institute (MNI) space using affine and non-linear transformations matching to the ICBM152 template (Fonov et al., 2009). The MR images in MNI space were then segmented into white matter, gray matter, and cerebrospinal fluid using a probabilistic atlas-based approach (Automatic Nonlinear Image Matching and Anatomical Labeling or ANIMAL) (Collins & Evans, 1997). The primary a priori regions of interest (ROIs) were the ventral limbic striatum (VST), associative striatum (AST) and somatosensory striatum (SMST), as defined by Mawlawi and colleagues (2001).

The dynamic PET images were reconstructed into 33 frames of progressively increasing duration. Images were then reconstructed using a 3D Ordinary-Poisson Ordered Subset Expectation-Maximization (OP-OSEM) algorithm (van Velden et al., 2009) including corrections applied for motion, random events, attenuation, scatter, decay, and intensity normalization. Finally, data collected during the 60-90-minute time frame after injection (binned into six 5-minute frames)

were averaged and reconstructed into static PET images. SUVs were calculated for each voxel from these static PET images, using the following formula:

$$SUV = \frac{radioactivity\ concentration\ (MBq/mL)}{\frac{injected\ dose\ (MBq)}{body\ weight\ (g)}}$$

For the a priori ROI analysis, masks applied to these static images were co-registered using transformations derived from the rigid-body transformation between the PET volume and native MR image concatenated with non-linear and affine transformations between MR and stereotaxic space. For the 2 subjects who were missing a T1-weighted MR image, only non-linear and affine transformations were used, and adjustments were made manually.

Mean regional SUVs for each ROI were then derived from these images in native PET space, calculated as the spatially weighted average of the regions across hemispheres based on the number of voxels. Mean regional SUVs were then normalized by the whole brain mean to calculate region-specific SUVRs. There was no difference in mean whole-brain SUV between the healthy controls and cocaine users (p=0.45).

Finally, an exploratory whole brain voxel-wise analysis of SUVR was performed between controls and cocaine users using SPM12 (described in Section 2.4. Statistical Analysis).

2.4. Statistical Analysis

All statistical analyses described below were conducted using SPSS27. Demographic, behavioural, and substance use-related measures were compared between the two groups using a series of Mann-Whitney U tests. A non-parametric test was selected given the non-normal

distribution of behavioural and self-reported substance use measures. Categorical variables, such as sex and smoking status, were compared using Fisher's exact test.

Potential group differences in VST, AST, and SMST SUVRs were tested for using a two-factor mixed-design analysis of variance (ANOVA), with ROI as the within-subject and cocaine use status as the between-subject variable. Sex was added as a covariate to account for potential sex differences in HDAC function as described in previous literature (Gilbert, Zürcher, Catanese, et al., 2019). Potential confounding effects by other behavioural measures were assessed using Spearman rank correlations. In the cocaine users, curve estimation regression analyses were performed between striatal SUVR and cocaine use parameters, namely lifetime number of occasions, monthly number of occasions during the past 90 days, and number of days since last use. To test if this effect is specific for cocaine use or also present for other substances, this analysis was repeated for monthly alcohol and cannabis use, as well as daily cigarette use, in the past 90 days, in participants who reported using these substances in the given time frame.

The hypothesis that striatal HDAC expression mediates the effect of early life adversity and cocaine use outcomes was tested using Hayes's simple mediator model with a binary dependent variable (Hayes, 2017). Bootstrapping was applied to test the significance of [^{11}C]Martinostat SUVR in each ROI as a mediator between lognormally transformed total CTQ scores and cocaine use group (HC vs. CU), with 1000 resamples and a confidence interval of 95%, using the PROCESS macro for SPSS (Hayes & Rockwood, 2017). This analysis was followed by a series of curve estimation regression analyses and non-parametric Spearman rank correlation tests between total CTQ scores and SUVR for each ROI to further explore the relationship between the two variables.

Finally, an exploratory, whole-brain voxel-wise group comparison between controls and cocaine users was conducted for [^{11}C]Martinostat SUVR using SPM12 with a two-sample test, unequal variance, a significance threshold of $t > 3.6$, and cluster-level correction of $P_{FWE} < .05$. Cluster-level analyses were based on a primary voxel level threshold of $p < .001$. Sex was added to the model as a covariate.

CHAPTER 3: RESEARCH FINDINGS

3.1. Comparison of Healthy Controls and Cocaine Users

3.1.1. Demographic and Behavioural Characteristics

Demographic information and behavioural participant metrics are presented in Table 1. The two groups (healthy controls, HC, n=13; cocaine users, CU, n=8) did not significantly differ in age (U=56.5 , p=.750), body mass index (U=73.0, p=.140), or sex distribution (p=.080). There were no statistically significant group differences in injected [^{11}C]Martinostat dose (U=57.5, p=.697), or specific activity at injection (U=72.0, p=.161).

Cocaine users, compared to control subjects, reported significantly higher current depression scores as measured by the BDI-II (U=80.5, p=.037), and higher early life adversity, as measured with the CTQ (U=81.0, p=.037). Conversely, the two groups did not report significantly different levels of state or trait anxiety, as measured by the STAI-S and STAI-T respectively. As expected, cocaine users reported higher levels of impulsivity than controls, as measured by both the BIS-11 (U=93.5, p=.001), and the SURPS impulsivity subscale (U=81.0, p=.037), whereas no significant differences were found in the SURPS sensation seeking, introversion/hopelessness, or anxiety sensitivity subscales (Table 1).

3.1.2. Substance Use Histories

Substance use-related participant metrics are presented in Table 2. All participants in both experimental groups reported consuming alcohol in their lifetime, and the proportion of healthy controls reporting alcohol consumption in the past 90 days did not significantly differ (p=.131). However, cocaine users reported higher lifetime alcohol use (U=87.5, p=.008), higher current

monthly frequency of alcohol use (U=93.5, p=.001), and higher AUDIT scores (U=98.0, p<.001). Similarly, the ratio of smokers to non-smokers was significantly higher in the cocaine user group than the control group (p=.047), and the average number of cigarettes smoked per day was significantly higher for cocaine users (U=81.0, p=.037). Cocaine users also reported higher lifetime cannabis use (U=95.0, p=.001), as well as higher current monthly cannabis use (U=85.0, p=.016). A few participants in both groups reported non-recreational lifetime use of opiates (morphine, oxycodone, or codeine) but the proportion of lifetime users or lifetime number of occasions did not significantly differ.

Cocaine users reported lifetime use of various other substances. With the exception of one, all subjects reported use of amphetamines, with four reporting relatively infrequent use in the past 90 days. Similarly, all subjects reported using 3,4-methylenedioxy-methamphetamine (MDMA, "Molly" or "ecstasy"), with only two reporting use in the past 90 days. Other reported substances included ketamine, phencyclidine (PCP or "angel dust"), psylocibin ("mushrooms") and other hallucinogens (including lysergic acid diethylamide [LSD or "acid"], *N*,*N*-dimethyltryptamine [DMT], 4-Bromo-2,5-dimethoxyphenethylamine [2-CB], and d-lysergic acid amide [LSA or ergine]), *gamma*-hydroxybutyric acid (GHB), salvia, benzodiazepines (only alprazolam), and alkyl nitrites ("poppers") (see Table 2).

3.2. Cocaine Use and Striatal HDAC Expression

3.2.1. Striatal [^{11}C]Martinostat SUVR in Cocaine Users and Healthy Controls

[^{11}C]Martinostat SUVRs were derived for the three functional ROIs, the ventral limbic striatum (VST), associative striatum (AST), and sensorimotor striatum (SMST) (see Figure 1A).

There was a significant main effect of the striatal ROI ($F(2, 38)=173.6$, $p<.001$). Pairwise comparisons confirmed that SUVR in SMST was higher than both in AST ($t(20)=3.74$, $p=.001$), and in VST ($t(20)=21.5$, $p<.001$), while SUVR in VST was lower than in AST ($t(20)=-26.2$, $p<.001$), meaning an overall higher expression of HDAC in dorsal regions of the striatum than the ventral striatum (see Figure 1B). As found previously (Gilbert, Zürcher, Catanese, et al., 2019), sex did not have a main effect on striatal SUVR ($F(1, 20)=1.71$, $p=.206$).

When comparing cocaine users to healthy controls, there was no significant main effect of group ($F(1, 19)=.009$, $p=.927$) or significant interaction between group and striatal ROI ($F(2, 38)=.306$, $p=.738$). Post-hoc independent t-tests with unequal variance confirmed that mean SUVR did not significantly differ between the two groups in VST ($t(19)=-.16$, $p=.878$), in AST ($t(17.8)=-.99$, $p=.337$), or in SMST ($t(16.6)=-.86$, $p=.400$) (see Figure 1B). No significant correlations were detected between striatal SUVR and behavioural measures that were significantly different between the two groups, namely depressive symptoms and impulsivity.

An exploratory unbiased voxel-wise analysis to evaluate relative [^{11}C]Martinostat SUVR differences between cocaine users and healthy controls across the whole brain identified 35 clusters with peak voxels above the height threshold ($t > 3.6$), but none survived the family-wise error correction ($P_{FWE} < .05$).

3.2.2. Striatal [^{11}C]Martinostat SUVR and Cocaine Use Variables

Among cocaine users, curve estimation analyses revealed a significant logarithmic relationship between striatal SUVR and some cocaine use variables. Current monthly cocaine use predicted SUVR in VST ($R^2=.63$, $F(1, 6)=10.1$, $p=.019$) (see Figure 2A) and AST ($R^2=.52$, $F(1, 6)=6.40$, $p=.045$) (see Figure 2B), but not in SMST ($R^2=.05$, $F(1, 6)=.281$, $p=.615$) (see Figure 2C).

Conversely, lifetime occasions of cocaine use did not significantly predict SUVR in any of the striatal regions. Time since last cocaine use also significantly predicted SUVR in VST (R^2=.64, F(1, 6)=10.6, p=.017) (see Figure 3A). This relationship between duration of abstinence and SUVR in AST showed a similar trend but the effect was not significant (R^2=.32, F(1, 6)=2.79, p=.146) (see Figure 3B), whereas the effect was not observed in SMST (R^2=.02, F(1, 6)=.129, p=.732) (see Figure 3C). No significant relationship was demonstrated between striatal SUVR and other substance use variables, namely current daily cigarette use, monthly alcohol use, monthly cannabis use, and monthly amphetamine use.

3.3. Early Life Adversity and Striatal HDAC Expression

Total CTQ scores were positively correlated with SUVR in AST (r_s(21)=.45, p=.040) (Figure 4B), but not in VST (r_s(21)=.28, p=.225) (Figure 4A), or in SMST (r_s(21)=.-.08, p=.738) (Figure 4C). Regression analyses revealed no linear or non-linear relationship between CTQ scores or SUVR in any striatal region. Similarly, investigating potential HDAC-mediation of the association between early life adversity and cocaine use group, neither CTQ scores (B=7.26, SE=4.19, p=.083), nor striatal SUVR in VST (B=-6.42, SE=12.7, p=.613) were significantly independently associated with cocaine use group. The results, which were similar for AST and SMST SUVR, did not support the mediational hypothesis.

CHAPTER 4: DISCUSSION

4.1. Increased Striatal HDAC Expression in Response to Cocaine Use

The current study constitutes, to our knowledge, the first investigation of *in vivo* HDAC expression in the human brain in a population of substance users. Concordant with findings in laboratory animals, the present results suggest that cocaine use leads to changes in histone acetylation within the striatum (Kumar et al., 2005; Levine et al., 2005, 2011; Renthal et al., 2009) likely as a consequence of increased class I HDAC activity (Malvaez et al., 2010; Kennedy et al., 2013; Romieu et al., 2008, 2011) and expression (Cassel et al., 2006; Host et al., 2011). The present study tested these hypotheses with PET [^{11}C]Martinostat neuroimaging.

4.1.1. Effects of Current Cocaine Use Frequency

The primary finding was that, among cocaine users, class I HDAC availability in VST and AST increased significantly with more frequent cocaine use (see Figure 2A and 2B). The association reflected a logarithmic relationship, such that striatal HDAC expression was higher among those who used cocaine more frequently, reaching a plateau when approaching daily use. This is consistent with findings from rodent models showing distinct striatal histone acetylation patterns for different cocaine administration regimens (Kumar et al., 2005; Wang et al., 2010). Among the class I HDAC enzymes targeted by [^{11}C]Martinostat, HDAC2 has been implicated specifically. Following repeated cocaine use in rats, HDAC2 mRNA expression has been shown to increase in both the ventral and dorsal striatum (Cassel et al., 2006; Host et al., 2011), which is in line with the current finding.

4.1.2. Transient Effects of Cocaine Use

In contrast to the association with recent cocaine use, striatal [^{11}C]Martinostat SUVR was not predicted by lifetime cocaine use, raising the possibility that cocaine's effects on HDAC1, 2, and 3 may be transient. This observation was supported by the finding that [^{11}C]Martinostat SUVR in VST was negatively predicted by duration of abstinence (see Figure 3A), with higher HDAC expression corresponding to more recent use, decreasing to the levels seen in stimulant drug-naïve healthy controls as the duration of abstinence increased. This apparent transient increase in HDAC expression could be observed in AST, albeit the effect did not attain statistical significance (see Figure 3B); however, the pattern was completely absent in SMST (see Figure 3C). While the lack of statistical significance might reflect low statistical power due to the modest sample size, this could also be the result of an amplified and more sustained increase in HDAC expression in VST than in the dorsal regions. This interpretation is supported by evidence that, in rats, two hours after a cocaine self-administration session, HDAC2 mRNA levels were increased 16-fold in the NAc, compared with 3-fold in dorsal aspects of the striatum (Host et al., 2011).

The temporal dynamics of histone acetylation patterns and involved enzymatic activities remain poorly understood. While its downstream effects on gene expression, likely through upregulation of transcription factors, involve long-lasting and even permanent changes in neural plasticity and function, histone acetylation itself is not known to be a persistent histone modification (Rogge & Wood, 2013), with chromatin-modifying enzymes generating a steady-level of histone acetylation through continuously occurring acetylation and deacetylation reactions in a dynamic equilibrium (Katan-Khaykovich & Struhl, 2002). Therefore, it is plausible that expression of HDAC may respond to environmental stimuli, such as cocaine, in an acute fashion. Supporting this interpretation, in mice, the increase in striatal HDAC2 mRNA expression

following repeated cocaine use was observed after 15 hours (Cassel et al., 2006), but not after 24 hours (Renthal et al., 2007). Although the *in vivo* timeline for these processes in the human brain remains unknown, the current finding suggests that cocaine intake may also lead to a temporary increase in class I HDAC expression in VST that is sustained for a few days. This could also explain why frequent cocaine use (such as weekly or semi-daily use) can have larger effects than more intermittent use (such as sporadic use over several months) on striatal HDAC expression, neurobiology and behaviour, as sustained histone acetylation by repeated cocaine exposure could have larger effects on gene expression (Rogge & Wood, 2013).

4.1.3. Comparison of Striatal Regions and Experimental Groups

The analysis of [^{11}C]Martinostat SUVR variation across striatal regions and experimental groups revealed that SUVR was highest in SMST and lowest in VST (see Figure 1). It was previously noted that, among 14 distinct regions in the human brain (excluding the NAc), [^{11}C]Martinostat SUVR was highest in the cerebellum and putamen (Wey et al., 2016). This is consistent with the current finding, as SMST constitutes the posterior putamen (Parent & Hazrati, 1995). However, this difference in HDAC expression between ventral and dorsal striatum had not been previously documented and might be driven by partial volume effects (Aston et al., 2002).

Contrary to the hypothesis, the results did not show a significant difference in striatal [^{11}C]Martinostat SUVR between cocaine users and stimulant-drug naïve controls in the current sample (see Figure 1B). Similarly, the exploratory voxel-wise whole brain analysis did not yield significantly different clusters between the two groups. However, the cocaine user group studied here included individuals reporting a wide range of lifetime and current use of cocaine. The significant positive relationship between current cocaine use frequency and striatal HDAC

expression suggests that a group difference may be observed between healthy controls and more frequent cocaine users, such as those diagnosed with a moderate or severe cocaine use disorder.

While the two groups did not differ in terms of age, body mass index, sex distribution, and a number of behavioural measures, cocaine users reported significantly higher current depression and impulsivity scores (see Table 1). Whereas impulsivity has not directly been linked with HDAC function previously, numerous findings have implicated a role for class I HDACs in the maintenance of depression-like behaviours in rodents (Covington et al., 2009; Tsankova et al., 2006; Uchida et al., 2011). Despite this, significant correlations with personality traits and depressive features were not observed in the current sample. Similarly, cocaine users reported higher amounts of lifetime and current alcohol, tobacco, and cannabis use than healthy controls (see Table 2). Curve estimation analyses did not reveal any significant relationship between striatal [^{11}C]Martinostat SUVR and these substance use measures. Together, these results suggest that, although we cannot unambiguously disentangle cause and effect, the observed changes to HDAC expression may be primarily driven by cocaine use.

4.2. Weak Evidence of HDAC-Mediation of Early Life Adversity on Cocaine Use

A second aspect of this study was investigating the epigenetic link between early life adversity and cocaine use. As a well-established environmental risk factor for addiction (Teixeira et al., 2017; Van Dam et al., 2014), early life adversity drives transcriptional changes in the mesocorticolimbic pathway that are sustained across the lifespan (Peña et al., 2017, 2019). In laboratory animals, early life chronic stress has been linked with changes in striatal class I HDAC function (Covington et al., 2009; Tesone-Coelho et al., 2013). Therefore, the secondary aim of the

study was to correlate striatal class I HDAC expression with early life adversity and to establish its role as a mediator of the effects of early life adversity on cocaine use outcomes.

In the current sample, a significant positive correlation was observed between CTQ scores and [^{11}C]Martinostat SUVR in AST (see Figure 4B). However, contrary to the finding in animals showing maternal separation-induced increases in basal HDAC2 mRNA levels in the adult rat NAc (Tesone-Coelho et al., 2013), this correlation did not attain statistical significance in VST, albeit a similar pattern of positive correlation could be observed (see Figure 4A). While this non-significance may reflect low statistical power, it might also hint at a similar transience in the effects of early life adversity on HDAC expression. In the rodent study, the effect was observed in adult rats (3 months of age), only 68 days after the maternal deprivation procedure (Tesone-Coelho et al., 2013). Therefore, in the current sample with a median age of 24, it is possible that effects of early life adversity on HDAC expression itself were not sustained for almost a decade in VST. As highlighted above, the temporal dynamics of HDAC activity are understudied and it is unclear through which mechanisms these seemingly transient changes in HDAC expression can be maintained in the rat NAc or the human AST following early life exposures to repeated stress.

It remains possible that early life adversity-induced changes in HDAC expression influence HDAC responses to subsequent stressors. However, it is important to note that chronic stress experienced during adulthood may have antagonistic effects. Adult mice exposed to chronic social defeat stress were shown to exhibit reduced HDAC2 mRNA levels in the NAc (Covington et al., 2009). This suggests that, in adults, the consequences of lifetime exposures to stress on HDAC function may be cumulative, complicating the observation of any direct effects of early life adversity. Seeing that the curve estimation did not reveal a significant trend for CTQ scores in the three regions, the findings prove inconclusive.

Cocaine users reported higher childhood trauma scores compared to healthy controls (see Table 1); however, in the mediation analysis, these scores did not significantly predict cocaine use group independently from striatal HDAC expression, suggesting that the effects of early life adversity may be obscured by overlapping effects of cocaine use. However, another possible reason for these results is that, in the combined sample, there were only 3 individuals reporting severe childhood trauma with a total CTQ score over 60 who may be qualitatively distinct from the rest of the sample, all of whom reported a score below 45 (see Figure 4), in terms of striatal HDAC function. Some epidemiological studies suggested that, while physical and sexual abuse predicted variables of substance and alcohol use, more prevalent adversities such as emotional neglect did not (Cheng & Lo, 2010; Galaif et al., 2001), highlighting potential differences in the effects of different types of early life exposures to stress. With no independent relationship between early life adversity and cocaine use, the current study could not provide evidence for the hypothesized mediatory role of class I HDACs.

4.3. Limitations and Future Directions

Class I HDACs are globally expressed in the brain and participate in various aspects of brain function, including glial lineage development, learning and memory, and, potentially, neuropsychiatric disease (Volmar & Wahlestedt, 2015). In the prefrontal cortex, deficient HDAC2 expression may underlie schizophrenia-induced cognitive impairment (Gilbert, Zürcher, Wu, et al., 2019), while some reports link elevated levels of HDAC1 to the cognitive phenotype of schizophrenia (Bahari-Javan et al., 2017; Jakovcevski et al., 2013; Sharma et al., 2008). In hippocampal regions, HDAC2 and 3 have been identified as negative regulators of synaptic plasticity and memory formation (Guan et al., 2009; Malvaez et al., 2013; McQuown et al., 2011;

Rogge et al., 2013) and some evidence suggests that HDAC1 activity facilitates fear memory extinction (Bahari-Javan et al., 2012). Both the loss of fronto-executive inhibitory control (Bolla et al., 1999; Ersche et al., 2013) and the hippocampus-dependent drug-context associations (Childress et al., 1999; Grant et al., 1996) have been implicated in the development and maintenance of cocaine addiction. Therefore, these diverse functions of different class I HDAC isoforms in different brain regions represent promising new directions in this line of research.

One limitation of the present study is that the [^{11}C]Martinostat SUVR values in the three striatal regions may potentially correspond to different subsets of class I HDACs engaged by the radiotracer, namely HDAC1, 2, 3, and, to a significantly lesser extent, HDAC6. That is, the outcome variable used throughout the study is an aggregate measure of class I HDAC expression, as opposed to representative of individual paralogs. This means that the observed effects of cocaine use or early life adversity on SUVR may reflect a mixture of opposing changes in expression of different HDAC isoforms and certain effects may be obscured if, for example, two isoforms respond in the opposite direction to environmental stimuli. Moreover, [^{11}C]Martinostat SUVR is not a direct measure of histone acetylation, but of the enzymes involved in the process, complicating speculations on the downstream effects of observed differences. Finally, while no significant off-target binding sites have been identified, Martinostat was shown to exhibit minimal binding *in vitro* to DAT, resulting in a 24% inhibition of the transporters (Wang et al., 2014). While this is smaller than the minimum 50% inhibition threshold of identifying a potential binding event, it leaves open the possibility for putative binding interactions.

The current study measured differences in [^{11}C]Martinostat uptake relative to the whole brain mean, i.e., SUVR, and not absolute uptake values. Therefore, the observed effects of cocaine on HDAC expression will need to be validated in future studies with arterial blood sampling in larger

sample sizes. In this relative measurement, identification of an appropriate reference region is of great importance; however, due to the ubiquity of class I HDAC expression in the brain, no particular region is suitable as a reference tissue with no radioligand binding or no spillover effects from adjacent tissues, falsely reducing the SUVR measure. While whole brain normalization allows controlling for inter-individual differences in global signal, potential radiotracer uptake differences in brain regions outside the striatum may reflect themselves in the outcome variable. Although no significant difference was observed in whole brain mean SUVs between the two groups and whole brain voxel-wise analysis did not yield any significantly different clusters, cocaine-induced differences in HDAC expression in other regions may be a potential confounder.

Due to the stringent criteria of participant recruitment, the analysis was conducted using a modest sample size. As described above, with the inclusion of more cocaine users – especially those with higher cocaine use frequencies – a group difference from stimulant-naïve controls could potentially be demonstrated, given the significant effect of current cocaine use frequency on striatal HDAC expression. Additionally, the current frequency of cocaine use and number of days since last use are highly intercorrelated variables in the current sample, complicating the determination of which variable drives the observed effect on striatal HDAC expression. With a larger sample of cocaine users, the interrelation of these measures could be more meaningfully explored using a multiple regression model.

Despite the fact that no correlation was detected in the current study between [^{11}C]Martinostat SUVR and alcohol, tobacco, cannabis, and amphetamine use measures, cocaine users also reported lifetime use of other stimulants, depressants, hallucinogens (psychedelics and dissociatives), and inhalants with varying levels of frequency (Table 2). There is some evidence in the literature suggesting alcohol (Caputi et al., 2015), tobacco (Castino et al., 2015),

methamphetamine (Martin et al., 2012; Torres et al., 2016), and amphetamine (Kim et al., 2008; Renthal et al., 2008) may induce changes in HDAC function and expression in the striatum and other brain regions. Therefore, potential confounding effects by drugs of abuse besides cocaine cannot be decisively ruled out. With a larger sample size, more robust statistical analyses could be performed, incorporating measures regarding the use of these substances as covariates. Alternatively, a future study could involve a multiple regression model including different substances as predictors could be used to contrast the effects of these substances.

Similar to non-cocaine drugs of abuse, sex has also been previously linked with differential HDAC function (Gilbert, Zürcher, Catanese, et al., 2019), in addition to differential effects of early life adversity (Hyman et al., 2006). Although sex distribution was not statistically different between the two groups and a difference in striatal HDAC expression between males and females was not demonstrated, sex was added as a covariate in the exploration of group differences between cocaine users and controls. Nonetheless, to effectively account for potential confounding effects of sex in the analysis, future studies could utilize a sex-balanced recruitment approach or designate experimental sub-groups based on sex.

The weak association between [^{11}C]Martinostat SUVR and severe childhood trauma could not be further investigated due to the modest sample size. With the inclusion of more subjects with high childhood trauma scores in both groups, a more robust mediation analysis could be performed. Similarly, with a sample where reporting of physical and sexual abuse is more prevalent, these subscales could be investigated as distinct measures, instead of a composite childhood trauma score.

Lastly, the interpretation of the results in regard to the effects of frequency and recency of cocaine use in this MSc thesis hinges on the current limited understanding of the temporal

dynamics of histone acetylation as a mechanism. This line of research would greatly benefit from either a longitudinal design with multiple timepoints or a time-sensitive approach towards duration of abstinence from stimulant drugs, in order to estimate the timescale of these effects in humans.

4.4. Conclusion

HDACs are increasingly being pursued as potential therapeutic targets in psychiatric illness and addiction, in light of findings from animal research demonstrating their role in the development and maintenance of pathological phenotypes. Therefore, it is imperative to study these mechanisms in living human brain. The findings described in this thesis comprise the first ever *in vivo* evidence of altered HDAC expression in response to cocaine use in the human striatum. Specifically, the study demonstrated that frequent cocaine use corresponds with increased class I HDAC expression in the ventral and parts of the dorsal striatum. The results suggest that, while these increases may be temporary, with more frequent exposure to cocaine, they may be sustained over longer periods of time. Further study of the temporal dynamics of HDAC activity are needed to obtain a fuller picture.

A second, and more tentatively identified finding was a correlation between early life adversity and HDAC expression in the dorsal striatum, raising the possibility that early life stress may exert long-lasting effects on brain HDAC function. However, the attempt to test the hypothesized mediatory role of striatal HDAC expression between early life adversity and cocaine use outcomes produced inconclusive results, potentially due to overlapping effects of stress and cocaine use. Future investigations will need to focus on a more stringent categorization of early

life adversity and take a deeper look into the mechanisms that maintain epigenetic markers of chronic stress and trauma across the lifespan.

Together, these studies benefit from the recent development of tools allowing us to characterize the link between early life experiences, epigenetic modifications, and enduring susceptibility to addictions in humans. By coupling these findings with behavioral, neurobiological, and gene editing studies in laboratory animals, we have an unprecedented opportunity to characterize drug use related molecular pathways to addiction, including potential therapeutic targets.

LITERATURE CITED

Adams, C. D., & Dickinson, A. (1981). Instrumental Responding following Reinforcer Devaluation. *The Quarterly Journal of Experimental Psychology Section B*, *33*(2b), 109–121. https://doi.org/10.1080/14640748108400816

Ad-Dab'bagh, Y., Lyttelton, O., Muehlboeck, J. S., Lepage, C., Einarson, D., Mok, K., ... & Evans, A. C. (2006, June). The CIVET image-processing environment: a fully automated comprehensive pipeline for anatomical neuroimaging research. In *Proceedings of the 12th annual meeting of the organization for human brain mapping* (Vol. 2266). Florence, Italy.

Antelman, S. M., Eichler, A. J., Black, C. A., & Kocan, D. (1980). Interchangeability of stress and amphetamine in sensitization. *Science, 207*(4428), 329-331. https://doi.org/10.1126/science.7188649

Aston, J. A., Cunningham, V. J., Asselin, M. C., Hammers, A., Evans, A. C., & Gunn, R. N. (2002). Positron emission tomography partial volume correction: estimation and algorithms. *Journal of Cerebral Blood Flow & Metabolism*, *22*(8), 1019-1034. https://doi.org/10.1097/00004647-200208000-00014

Baarendse, P. J., Limpens, J. H., & Vanderschuren, L. J. (2013). Disrupted social development enhances the motivation for cocaine in rats. *Psychopharmacology*, *231*(8), 1695–1704. https://doi.org/10.1007/s00213-013-3362-8

Bagot, R. C. (2014). Epigenetic signaling in psychiatric disorders: stress and depression. *Dialogues in Clinical Neuroscience, 16*(3), 281–295. https://doi.org/10.31887/dcns.2014.16.3/rbagot

Bahari-Javan, S., Maddalena, A., Kerimoglu, C., Wittnam, J., Held, T., Bähr, M., Burkhardt, S., Delalle, I., Kügler, S., Fischer, A., & Sananbenesi, F. (2012). HDAC1 regulates fear extinction in mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 32*(15), 5062–5073. https://doi.org/10.1523/jneurosci.0079-12.2012

Bahari-Javan, S., Varbanov, H., Halder, R., Benito, E., Kaurani, L., Burkhardt, S., Anderson-Schmidt, H., Anghelescu, I., Budde, M., Stilling, R. M., Costa, J., Medina, J., Dietrich, D. E., Figge, C., Folkerts, H., Gade, K., Heilbronner, U., Koller, M., Konrad, C., Nussbeck, S. Y., … Fischer, A. (2017). HDAC1 links early life stress to schizophrenia-like phenotypes. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(23), E4686–E4694. https://doi.org/10.1073/pnas.1613842114

Beck, A. T., Steer, R. A., Ball, R., & Ranieri, W. (1996). Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *Journal of personality assessment, 67*(3), 588–597. https://doi.org/10.1207/s15327752jpa6703_13

Belin, D., & Everitt, B. J. (2008). Cocaine Seeking Habits Depend upon Dopamine-Dependent Serial Connectivity Linking the Ventral with the Dorsal Striatum. *Neuron, 57*(3), 432–441. https://doi.org/10.1016/j.neuron.2007.12.019

Bernstein, D. P., Stein, J. A., Newcomb, M. D., Walker, E., Pogge, D., Ahluvalia, T., ... & Zule, W. (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child abuse & neglect*, *27*(2), 169-190. https://doi.org/10.1016/s0145-2134(02)00541-0

Bernstein, D., & Fink, L. (1998). *Childhood trauma questionnaire: A retrospective self-report*. The San Antonio: Psychological Corporation.

Berridge, K. C. (2017). Is addiction a brain disease? *Neuroethics*, *10*(1), 29-33. https://doi.org/10.1007/s12152-016-9286-3

Bierut, L. J., Dinwiddie, S. H., Begleiter, H., Crowe, R. R., Hesselbrock, V., Nurnberger, J. I., ... & Reich, T. (1998). Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking: a report from the Collaborative Study on the Genetics of Alcoholism. *Archives of general psychiatry*, *55*(11), 982-988. https://doi.org/10.1001/archpsyc.55.11.982

Blaze, J., & Roth, T. L. (2013). Exposure to caregiver maltreatment alters expression levels of epigenetic regulators in the medial prefrontal cortex. *International Journal of*

Developmental Neuroscience, *31*(8), 804–810. https://doi.org/10.1016/j.ijdevneu.2013.10.001

Boileau, I., Dagher, A., Leyton, M., Gunn, R. N., Baker, G. B., Diksic, M., & Benkelfat, C. (2006). Modeling sensitization to stimulants in humans: an [11C] raclopride/positron emission tomography study in healthy men. *Archives of general psychiatry*, *63*(12), 1386-1395. https://doi.org/10.1001/archpsyc.63.12.1386

Boileau, I., Dagher, A., Leyton, M., Welfeld, K., Booij, L., Diksic, M., & Benkelfat, C. (2007). Conditioned dopamine release in humans: a positron emission tomography [11C] raclopride study with amphetamine. *Journal of Neuroscience*, *27*(15), 3998-4003. https://doi.org/10.1523/jneurosci.4370-06.2007

Bolla, K. I., Rothman, R., & Cadet, J. L. (1999). Dose-related neurobehavioral effects of chronic cocaine use. *The Journal of neuropsychiatry and clinical neurosciences*, *11*(3), 361–369. https://doi.org/10.1176/jnp.11.3.361

Booij, L., Welfeld, K., Leyton, M., Dagher, A., Boileau, I., Sibon, I., … Benkelfat, C. (2016). Dopamine cross-sensitization between psychostimulant drugs and stress in healthy male volunteers. *Translational Psychiatry*, *6*(2). https://doi.org/10.1038/tp.2016.6

Brake, W. G., Zhang, T. Y., Diorio, J., Meaney, M. J., & Gratton, A. (2004). Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *European Journal of Neuroscience*, *19*(7), 1863–1874. https://doi.org/10.1111/j.1460-9568.2004.03286.x

Canadian Substance Use Costs and Harms Scientific Working Group. (2020). *Canadian substance use costs and harms: 2015-2017 [Internet]*. Ottawa, ON: Canadian Institute for Substance Use Research and the Canadian Centre on Substance Use and Addiction. Available from: https://csuch.ca/en/report/.

Caputi, F. F., Palmisano, M., D'Addario, C., Candeletti, S., & Romualdi, P. (2015). Effects of acute ethanol exposure on class I HDACs family enzymes in wild-type and BDNF(+/-) mice. *Drug and alcohol dependence*, *155*, 68–75. https://doi.org/10.1016/j.drugalcdep.2015.08.015

Cassel, S., Carouge, D., Gensburger, C., Anglard, P., Burgun, C., Dietrich, J.-B., … Zwiller, J. (2006). Fluoxetine and Cocaine Induce the Epigenetic Factors MeCP2 and MBD1 in Adult Rat Brain. *Molecular Pharmacology*, *70*(2), 487–492. https://doi.org/10.1124/mol.106.022301

Castino, M. R., Cornish, J. L., & Clemens, K. J. (2015). Inhibition of histone deacetylases facilitates extinction and attenuates reinstatement of nicotine self-administration in rats. *PloS one*, *10*(4), e0124796. https://doi.org/10.1371/journal.pone.0124796

Cheng, T. C., & Lo, C. C. (2010). Drug use among maltreated adolescents receiving child welfare services. *Children and Youth Services Review, 32*(12), 1735–1739. https://doi.org/10.1016/j.childyouth.2010.07.017

Childress, A. R., Mozley, P. D., McElgin, W., Fitzgerald, J., Reivich, M., & O'Brien, C. P. (1999). Limbic activation during cue-induced cocaine craving. *The American journal of psychiatry*, *156*(1), 11–18. https://doi.org/10.1176/ajp.156.1.11

Collins, D. L., & Evans, A. C. (1997). Animal: validation and applications of nonlinear registration-based segmentation. *International journal of pattern recognition and artificial intelligence, 11*(08), 1271-1294. https://doi.org/10.1142/S0218001497000597

Covington, H. E., 3rd, Maze, I., LaPlant, Q. C., Vialou, V. F., Ohnishi, Y. N., Berton, O., Fass, D. M., Renthal, W., Rush, A. J., 3rd, Wu, E. Y., Ghose, S., Krishnan, V., Russo, S. J., Tamminga, C., Haggarty, S. J., & Nestler, E. J. (2009). Antidepressant actions of histone deacetylase inhibitors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(37), 11451–11460. https://doi.org/10.1523/JNEUROSCI.1758-09.2009

Cox, S. M., Yau, Y., Larcher, K., Durand, F., Kolivakis, T., Delaney, J. S., ... & Leyton, M. (2017). Cocaine cue-induced dopamine release in recreational cocaine users. *Scientific reports*, *7*(1), 1-5. https://doi.org/10.1038/srep46665

Dallman, M. F., Akana, S. F., Bradbury, M. J., Strack, A. M., Hanson, E. S., & Scribner, K. A. (1994). Regulation of the hypothalamo-pituitary-adrenal axis during stress: feedback, facilitation and feeding. *Seminars in Neuroscience*, *6*(4), 205–213. https://doi.org/10.1006/smns.1994.1027

Daunais, J. B., Roberts, D. C., & McGinty, J. F. (1993). Cocaine self-administration increases preprodynorphin, but not c-fos, mRNA in rat striatum. *NeuroReport*, *4*(5), 543–546. https://doi.org/10.1097/00001756-199305000-00020

de Wit, H., & Wise, R. A. (1977). Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide, but not with the noradrenergic blockers phentolamine or phenoxybenzamine. *Canadian Journal of Psychology/Revue canadienne de psychologie*, *31*(4), 195–203. https://doi.org/10.1037/h0081662

Dickinson, A. (1985). Actions and habits: the development of behavioural autonomy. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, *308*(1135), 67–78. https://doi.org/10.1098/rstb.1985.0010

Doherty, M. D., & Gratton, A. (1992). High-speed chronoamperometric measurements of mesolimbic and nigrostriatal dopamine release associated with repeated daily stress. *Brain Research*, *586*(2), 295–302. https://doi.org/10.1016/0006-8993(92)91639-v

Donovan, L. L., Magnussen, J. H., Dyssegaard, A., Lehel, S., Hooker, J. M., Knudsen, G. M., & Hansen, H. D. (2020). Imaging HDACs in vivo: cross-validation of the [11C] Martinostat radioligand in the pig brain. *Molecular imaging and biology*, *22*(3), 569-577. https://doi.org/10.1007/s11307-019-01403-9

Ersche, K. D., Williams, G. B., Robbins, T. W., & Bullmore, E. T. (2013). Meta-analysis of structural brain abnormalities associated with stimulant drug dependence and neuroimaging of addiction vulnerability and resilience. *Current opinion in neurobiology*, *23*(4), 615–624. https://doi.org/10.1016/j.conb.2013.02.017

Espejo, E. P., Hammen, C. L., Connolly, N. P., Brennan, P. A., Najman, J. M., & Bor, W. (2007). Stress sensitization and adolescent depressive severity as a function of childhood adversity: a link to anxiety disorders. *Journal of abnormal child psychology*, *35*(2), 287-299. https://doi.org/10.1007/s10802-006-9090-3

Everitt, B. J., & Robbins, T. W. (2016). Drug Addiction: Updating Actions to Habits to Compulsions Ten Years On. *Annual Review of Psychology*, *67*(1), 23–50. https://doi.org/10.1146/annurev-psych-122414-033457

Flak, J. N., Solomon, M. B., Jankord, R., Krause, E. G., & Herman, J. P. (2012). Identification of chronic stress-activated regions reveals a potential recruited circuit in rat brain. *European Journal of Neuroscience*, *36*(4), 2547-2555. https://doi.org/10.1111/j.1460-9568.2012.08161.x

Fonov, V., Evans, A. C., Botteron, K., Almli, C. R., McKinstry, R. C., & Collins, D. L. (2011). Unbiased average age-appropriate atlases for pediatric studies. *NeuroImage*, *54*(1), 313–327. https://doi.org/10.1016/j.neuroimage.2010.07.033

Fosnocht, A. Q., Lucerne, K. E., Ellis, A. S., Olimpo, N. A., & Briand, L. A. (2019). Adolescent social isolation increases cocaine seeking in male and female mice. *Behavioural brain research*, *359*, 589-596. https://doi.org/10.1101/320648

Galaif, E. R., Stein, J. A., Newcomb, M. D., & Bernstein, D. P. (2001). Gender differences in the prediction of problem alcohol use in adulthood: exploring the influence of family factors and childhood maltreatment. *Journal of studies on alcohol*, *62*(4), 486–493. https://doi.org/10.15288/jsa.2001.62.486

Gilbert, T. M., Zürcher, N. R., Catanese, M. C., Tseng, C. E. J., Di Biase, M. A., Lyall, A. E., ... & Hooker, J. M. (2019). Neuroepigenetic signatures of age and sex in the living human brain. *Nature communications*, *10*(1), 1-9. https://doi.org/10.1038/s41467-019-11031-0

Gilbert, T. M., Zürcher, N. R., Wu, C. J., Bhanot, A., Hightower, B. G., Kim, M., ... & Hooker, J. M. (2019). PET neuroimaging reveals histone deacetylase dysregulation in schizophrenia. *The Journal of clinical investigation*, *129*(1), 364-372. https://doi.org/10.1172/JCI123743

Giuliano, C., Belin, D., & Everitt, B. J. (2019). Compulsive alcohol seeking results from a failure to disengage dorsolateral striatal control over behavior. *Journal of Neuroscience*, *39*(9), 1744-1754. https://doi.org/10.1523/jneurosci.2615-18.2018

Goldman, D., Oroszi, G., & Ducci, F. (2005). The genetics of addictions: uncovering the genes. *Nature Reviews Genetics*, *6*(7), 521-532. https://doi.org/10.1038/nrg1635

Grant, S., London, E. D., Newlin, D. B., Villemagne, V. L., Liu, X., Contoreggi, C., Phillips, R. L., Kimes, A. S., & Margolin, A. (1996). Activation of memory circuits during cue-elicited cocaine craving. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(21), 12040–12045. https://doi.org/10.1073/pnas.93.21.12040

Gratton, A., Hoffer, B. J., & Gerhardt, G. A. (1988). Effects of electrical stimulation of brain reward sites on release of dopamine in rat: an in vivo electrochemical study. *Brain research bulletin*, *21*(2), 319-324. https://doi.org/10.1016/0361-9230(88)90247-x

Guan, J. S., Haggarty, S. J., Giacometti, E., Dannenberg, J. H., Joseph, N., Gao, J., ... & Tsai, L. H. (2009). HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature*, *459*(7243), 55-60. https://doi.org/10.1038/nature07925

Hayes, A. F. (2017). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach*. Guilford Press.

Hayes, A. F., & Rockwood, N. J. (2017). Regression-based statistical mediation and moderation analysis in clinical research: Observations, recommendations, and implementation. *Behaviour research and therapy*, *98*, 39-57. https://doi.org/10.1016/j.brat.2016.11.001

Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology*, *33*(6), 693-710. https://doi.org/10.1016/j.psyneuen.2008.03.008.

Hendricks, J. A., Keliher, E. J., Marinelli, B., Reiner, T., Weissleder, R., & Mazitschek, R. (2011). In vivo PET imaging of histone deacetylases by 18F-suberoylanilide hydroxamic acid (18F-SAHA). *Journal of medicinal chemistry*, *54*(15), 5576-5582. https://doi.org/10.1021/jm200620f

Hitchcock, L. N., Raybuck, J. D., Wood, M. A., & Lattal, K. M. (2019). Effects of a histone deacetylase 3 inhibitor on extinction and reinstatement of cocaine self-administration in rats. *Psychopharmacology*, *236*(1), 517–529. https://doi.org/10.1007/s00213-018-5122-2

Honegger, K., & de Bivort, B. (2018). Stochasticity, individuality and behavior. *Current Biology*, *28*(1), R8-R12. https://doi.org/10.1016/j.cub.2017.11.058

Hooker, J. M., Kim, S. W., Alexoff, D., Xu, Y., Shea, C., Reid, A., ... & Fowler, J. S. (2010). Histone deacetylase inhibitor MS-275 exhibits poor brain penetration: pharmacokinetic studies of [11C] MS-275 using positron emission tomography. *ACS chemical neuroscience*, *1*(1), 65-73. https://doi.org/10.1021/cn9000268

Hope, B., Kosofsky, B., Hyman, S. E., & Nestler, E. J. (1992). Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. *Proceedings of the National Academy of Sciences*, *89*(13), 5764–5768. https://doi.org/10.1073/pnas.89.13.5764

Host, L., Dietrich, J.-B., Carouge, D., Aunis, D., & Zwiller, J. (2011). Cocaine self-administration alters the expression of chromatin-remodelling proteins; modulation by histone deacetylase inhibition. *Journal of Psychopharmacology*, *25*(2), 222–229. https://doi.org/10.1177/0269881109348173

Hyman, S. M., Garcia, M., & Sinha, R. (2006). Gender specific associations between types of childhood maltreatment and the onset, escalation and severity of substance use in cocaine dependent adults. *The American journal of drug and alcohol abuse*, *32*(4), 655–664. https://doi.org/10.1080/10623320600919193

Ieraci, A., Mallei, A., Musazzi, L., & Popoli, M. (2015). Physical exercise and acute restraint stress differentially modulate hippocampal brain-derived neurotrophic factor transcripts and epigenetic mechanisms in mice. *Hippocampus*, *25*(11), 1380–1392. https://doi.org/10.1002/hipo.22458

Ito, R., Dalley, J. W., Howes, S. R., Robbins, T. W., & Everitt, B. J. (2000). Dissociation in Conditioned Dopamine Release in the Nucleus Accumbens Core and Shell in Response to Cocaine Cues and during Cocaine-Seeking Behavior in Rats. *The Journal of Neuroscience*, *20*(19), 7489–7495. https://doi.org/10.1523/jneurosci.20-19-07489.2000

Ito, R., Dalley, J. W., Robbins, T. W., & Everitt, B. J. (2002). Dopamine Release in the Dorsal Striatum during Cocaine-Seeking Behavior under the Control of a Drug-Associated Cue. *The Journal of Neuroscience*, *22*(14), 6247–6253. https://doi.org/10.1523/jneurosci.22-14-06247.2002

Itzhak, Y., Liddie, S., & Anderson, K. L. (2013). Sodium butyrate-induced histone acetylation strengthens the expression of cocaine-associated contextual memory. *Neurobiology of Learning and Memory*, *102*, 34–42. https://doi.org/10.1016/j.nlm.2013.03.007

Jafari, R., Almqvist, H., Axelsson, H., Ignatushchenko, M., Lundbäck, T., Nordlund, P., & Molina, D. M. (2014). The cellular thermal shift assay for evaluating drug target interactions in cells. *Nature Protocols*, *9*(9), 2100–2122. https://doi.org/10.1038/nprot.2014.138

Jakovcevski, M., Bharadwaj, R., Straubhaar, J., Gao, G., Gavin, D. P., Jakovcevski, I., Mitchell, A. C., & Akbarian, S. (2013). Prefrontal cortical dysfunction after overexpression of histone deacetylase 1. *Biological psychiatry*, *74*(9), 696–705. https://doi.org/10.1016/j.biopsych.2013.03.020

Jarmasz, J. S., Stirton, H., Davie, J. R., & Del Bigio, M. R. (2019). DNA methylation and histone post-translational modification stability in post-mortem brain tissue. *Clinical Epigenetics*, *11*(1). https://doi.org/10.1186/s13148-018-0596-7

Jeste, D. V., Palmer, B. W., Appelbaum, P. S., Golshan, S., Glorioso, D., Dunn, L. B., Kim, K., Meeks, T., & Kraemer, H. C. (2007). A new brief instrument for assessing decisional capacity for clinical research. *Archives of general psychiatry*, 64(8), 966–974. https://doi.org/10.1001/archpsyc.64.8.966

Kalivas, P. W., & Stewart, J. (1991). Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Research Reviews*, *16*(3), 223–244. https://doi.org/10.1016/0165-0173(91)90007-u

Katan-Khaykovich, Y., & Struhl, K. (2002). Dynamics of global histone acetylation and deacetylation in vivo: rapid restoration of normal histone acetylation status upon removal of

activators and repressors. *Genes & development*, *16*(6), 743–752. https://doi.org/10.1101/gad.967302

Kendler, K. S., Sundquist, K., Ohlsson, H., Palmér, K., Maes, H., Winkleby, M. A., & Sundquist, J. (2012). Genetic and familial environmental influences on the risk for drug abuse: a national Swedish adoption study. *Archives of general psychiatry*, *69*(7), 690-697. https://doi.org/10.1001/archgenpsychiatry.2011.2112

Kennedy, P. J., Feng, J., Robison, A. J., Maze, I., Badimon, A., Mouzon, E., … Nestler, E. J. (2013). Class I HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. *Nature Neuroscience*, *16*(4), 434–440. https://doi.org/10.1038/nn.3354

Kikusui, T., Faccidomo, S., & Miczek, K. A. (2005). Repeated maternal separation: differences in cocaine-induced behavioral sensitization in adult male and female mice. *Psychopharmacology*, *178*(2), 202-210. https://doi.org/10.1007/s00213-004-1989-1

Kilgore, M., Miller, C. A., Fass, D. M., Hennig, K. M., Haggarty, S. J., Sweatt, J. D., & Rumbaugh, G. (2010). Inhibitors of Class 1 Histone Deacetylases Reverse Contextual Memory Deficits in a Mouse Model of Alzheimer's Disease. *Neuropsychopharmacology*, *35*(4), 870–880. https://doi.org/10.1038/npp.2009.197

Kim, S. W., Hooker, J. M., Otto, N., Win, K., Muench, L., Shea, C., ... & Fowler, J. S. (2013). Whole-body pharmacokinetics of HDAC inhibitor drugs, butyric acid, valproic acid and 4-phenylbutyric acid measured with carbon-11 labeled analogs by PET. *Nuclear medicine and biology*, *40*(7), 912-918. https://doi.org/10.1016/j.nucmedbio.2013.06.007

Kim, W. Y., Kim, S., & Kim, J. H. (2008). Chronic microinjection of valproic acid into the nucleus accumbens attenuates amphetamine-induced locomotor activity. *Neuroscience letters*, *432*(1), 54–57. https://doi.org/10.1016/j.neulet.2007.12.005

Kouzarides, T. (2007). Chromatin Modifications and Their Function. *Cell*, *128*(4), 693–705. https://doi.org/10.1016/j.cell.2007.02.005

Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., ... & Nestler, E. J. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*, *131*(2), 391-404. https://doi.org/10.1016/j.cell.2007.09.018

Kuhar, M. J., Ritz, M. C., & Sharkey, J. (1988). Cocaine receptors on dopamine transporters mediate cocaine-reinforced behavior. *NIDA Res Monogr*, *88*, 14-22.

Kumar, A., Choi, K.-H., Renthal, W., Tsankova, N. M., Theobald, D. E. H., Truong, H.-T., … Nestler, E. J. (2005). Chromatin Remodeling Is a Key Mechanism Underlying Cocaine-Induced Plasticity in Striatum. *Neuron*, *48*(2), 303–314. https://doi.org/10.1016/j.neuron.2005.09.023

LeBlanc, K. H., Maidment, N. T., & Ostlund, S. B. (2013). Repeated Cocaine Exposure Facilitates the Expression of Incentive Motivation and Induces Habitual Control in Rats. *PLoS ONE*, *8*(4). https://doi.org/10.1371/journal.pone.0061355

Lehrmann, E., Afanador, Z. R., Deep-Soboslay, A., Gallegos, G., Darwin, W. D., Lowe, R. H., … Freed, W. J. (2008). Postmortem diagnosis and toxicological validation of illicit substance use. *Addiction Biology*, *13*(1), 105–117. https://doi.org/10.1111/j.1369-1600.2007.00085.x

Levine, A. A., Guan, Z., Barco, A., Xu, S., Kandel, E. R., & Schwartz, J. H. (2005). CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. *Proceedings of the National Academy of Sciences*, *102*(52), 19186–19191. https://doi.org/10.1073/pnas.0509735102

Levine, A., Huang, Y., Drisaldi, B., Griffin, E. A., Jr, Pollak, D. D., Xu, S., Yin, D., Schaffran, C., Kandel, D. B., & Kandel, E. R. (2011). Molecular mechanism for a gateway drug: epigenetic changes initiated by nicotine prime gene expression by cocaine. Science translational medicine, 3(107), 107ra109. https://doi.org/10.1126/scitranslmed.3003062

Levine, A., Worrell, T. R., Zimnisky, R., & Schmauss, C. (2012). Early life stress triggers sustained changes in histone deacetylase expression and histone H4 modifications that alter

responsiveness to adolescent antidepressant treatment. Neurobiology of disease, 45(1), 488–498. https://doi.org/10.1016/j.nbd.2011.09.005

Leyton, M., & Stewart, J. (1990). Preexposure to foot-shock sensitizes the locomotor response to subsequent systemic morphine and intra-nucleus accumbens amphetamine. *Pharmacology Biochemistry and Behavior*, *37*(2), 303–310. https://doi.org/10.1016/0091-3057(90)90339-j

Leyton, M., & Vezina, P. (2014). Dopamine ups and downs in vulnerability to addictions: a neurodevelopmental model. *Trends in Pharmacological Sciences*, *35*(6), 268–276. https://doi.org/10.1016/j.tips.2014.04.002

Lo Iacono, L., Valzania, A., Visco-Comandini, F., Aricò, E., Viscomi, M. T., Castiello, L., … Carola, V. (2016). Social threat exposure in juvenile mice promotes cocaine-seeking by altering blood clotting and brain vasculature. *Addiction Biology*, *22*(4), 911–922. https://doi.org/10.1111/adb.12373

Lüscher, C. (2016). The emergence of a circuit model for addiction. *Annual review of neuroscience*, *39*, 257-276. https://doi.org/10.1146/annurev-neuro-070815-013920

Lüscher, C. (2016). The emergence of a circuit model for addiction. *Annual review of neuroscience*, *39*, 257-276. https://doi.org/10.1146/annurev-neuro-070815-013920

Lüscher, C., & Ungless, M. A. (2006). The mechanistic classification of addictive drugs. *PLoS Med*, *3*(11), e437. https://doi.org/10.1371/journal.pmed.0030437

Madsen, H., Brown, R., & Lawrence, A. (2012). Neuroplasticity in addiction: cellular and transcriptional perspectives. *Frontiers in molecular neuroscience*, *5*, 99. https://doi.org/10.3389/fnmol.2012.00099

Maldonado, R., Robledo, P., Chover, A. J., Caine, S. B., & Koob, G. F. (1993). D1 dopamine receptors in the nucleus accumbens modulate cocaine self-administration in the rat. *Pharmacology Biochemistry and Behavior*, *45*(1), 239-242. https://doi.org/10.1016/0091-3057(93)90112-7

Malvaez, M., McQuown, S. C., Rogge, G. A., Astarabadi, M., Jacques, V., Carreiro, S., … Wood, M. A. (2013). HDAC3-selective inhibitor enhances extinction of cocaine-seeking behavior in a persistent manner. *Proceedings of the National Academy of Sciences*, *110*(7), 2647–2652. https://doi.org/10.1073/pnas.1213364110

Malvaez, M., Sanchis-Segura, C., Vo, D., Lattal, K. M., & Wood, M. A. (2010). Modulation of Chromatin Modification Facilitates Extinction of Cocaine-Induced Conditioned Place Preference. *Biological Psychiatry*, *67*(1), 36–43. https://doi.org/10.1016/j.biopsych.2009.07.032

Martin, T. A., Jayanthi, S., McCoy, M. T., Brannock, C., Ladenheim, B., Garrett, T., … Cadet, J. L. (2012). Methamphetamine Causes Differential Alterations in Gene Expression and Patterns of Histone Acetylation/Hypoacetylation in the Rat Nucleus Accumbens. *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0034236

Matuszewich, L., Carter, S., Anderson, E. M., Friedman, R. D., & McFadden, L. M. (2014). Persistent behavioral and neurochemical sensitization to an acute injection of methamphetamine following unpredictable stress. *Behavioural Brain Research*, *272*, 308–313. https://doi.org/10.1016/j.bbr.2014.07.013

Mawlawi, O., Martinez, D., Slifstein, M., Broft, A., Chatterjee, R., Hwang, D. R., ... & Laruelle, M. (2001). Imaging human mesolimbic dopamine transmission with positron emission tomography: I. Accuracy and precision of D2 receptor parameter measurements in ventral striatum. *Journal of Cerebral Blood Flow & Metabolism*, *21*(9), 1034-1057. https://doi.org/10.1097/00004647-200109000-00002

McClung, C. A., & Nestler, E. J. (2003). Regulation of gene expression and cocaine reward by CREB and ΔFosB. *Nature Neuroscience*, *6*(11), 1208–1215. https://doi.org/10.1038/nn1143

McGregor, A., & Roberts, D. C. (1993). Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. *Brain research*, *624*(1-2), 245-252. https://doi.org/10.1016/0006-8993(93)90084-z

McLaughlin, K. A., Colich, N. L., Rodman, A. M., & Weissman, D. G. (2020). Mechanisms linking childhood trauma exposure and psychopathology: a transdiagnostic model of risk and resilience. *BMC medicine*, *18*, 1-11. https://doi.org/10.1186/s12916-020-01561-6

McQuown, S. C., Barrett, R. M., Matheos, D. P., Post, R. J., Rogge, G. A., Alenghat, T., Mullican, S. E., Jones, S., Rusche, J. R., Lazar, M. A., & Wood, M. A. (2011). HDAC3 is a critical negative regulator of long-term memory formation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *31*(2), 764–774. https://doi.org/10.1523/jneurosci.5052-10.2011

Meaney, M. J., Brake, W., & Gratton, A. (2002). Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology*, *27*(1-2), 127–138. https://doi.org/10.1016/s0306-4530(01)00040-3

Merrick, M. T., Ports, K. A., Ford, D. C., Afifi, T. O., Gershoff, E. T., & Grogan-Kaylor, A. (2017). Unpacking the impact of adverse childhood experiences on adult mental health. *Child abuse & neglect*, *69*, 10-19. https://doi.org/10.1016/j.chiabu.2017.03.016

Mews, P., & Calipari, E. S. (2017). Cross-talk between the epigenome and neural circuits in drug addiction. *Progress in brain research*, *235*, 19-63. https://doi.org/10.1016/bs.pbr.2017.08.012

Moloney, R. D., Stilling, R. M., Dinan, T. G., & Cryan, J. F. (2015). Early-life stress-induced visceral hypersensitivity and anxiety behavior is reversed by histone deacetylase inhibition. *Neurogastroenterology & Motility*, *27*(12), 1831–1836. https://doi.org/10.1111/nmo.12675

Murray, J. E., Belin, D., & Everitt, B. J. (2012). Double Dissociation of the Dorsomedial and Dorsolateral Striatal Control Over the Acquisition and Performance of Cocaine Seeking. *Neuropsychopharmacology*, *37*(11), 2456–2466. https://doi.org/10.1038/npp.2012.104

Nestler, E. J. (2001). Molecular basis of long-term plasticity underlying addiction. *Nature Reviews Neuroscience*, *2*(2), 119–128. https://doi.org/10.1038/35053570

Nestler, E. J. (2005). Is there a common molecular pathway for addiction?. Nature neuroscience, 8(11), 1445-1449. https://doi.org/10.1038/nn1578

Nestler, E. J., & Lüscher, C. (2019). The Molecular Basis of Drug Addiction: Linking Epigenetic to Synaptic and Circuit Mechanisms. *Neuron*, *102*(1), 48–59. https://doi.org/10.1016/j.neuron.2019.01.016

Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of comparative and physiological psychology*, *47*(6), 419. https://doi.org/10.1037/h0058775

Parent, A., & Hazrati, L. N. (1995). Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain research reviews*, *20*(1), 91-127. https://doi.org/10.1016/0165-0173(94)00007-c

Pascoli, V., Terrier, J., Hiver, A., & Lüscher, C. (2015). Sufficiency of mesolimbic dopamine neuron stimulation for the progression to addiction. *Neuron*, *88*(5), 1054-1066. https://doi.org/10.1016/j.neuron.2015.10.017

Patton, J. H., Stanford, M. S., & Barratt, E. S. (1995). Factor structure of the Barratt impulsiveness scale. *Journal of clinical psychology*, *51*(6), 768–774. https://doi.org/10.1002/1097-4679(199511)51:6<768::aid-jclp2270510607>3.0.co;2-1

Peña, C. J., Kronman, H. G., Walker, D. M., Cates, H. M., Bagot, R. C., Purushothaman, I., ... & Nestler, E. J. (2017). Early life stress confers lifelong stress susceptibility in mice via ventral tegmental area OTX2. *Science*, *356*(6343), 1185-1188. https://doi.org/10.1126/science.aan4491

Peña, C. J., Smith, M., Ramakrishnan, A., Cates, H. M., Bagot, R. C., Kronman, H. G., ... & Nestler, E. J. (2019). Early life stress alters transcriptomic patterning across reward circuitry in male and female mice. *Nature communications*, *10*(1), 1-13. https://doi.org/10.1038/s41467-019-13085-6

Pokholok, D. K., Harbison, C. T., Levine, S., Cole, M., Hannett, N. M., Lee, T. I., … Young, R. A. (2005). Genome-wide Map of Nucleosome Acetylation and Methylation in Yeast. *Cell*, *122*(4), 517–527. https://doi.org/10.1016/j.cell.2005.06.026

Renthal, W., Kumar, A., Xiao, G., Wilkinson, M., Covington, H. E., Maze, I., … Nestler, E. J. (2009). Genome-wide Analysis of Chromatin Regulation by Cocaine Reveals a Role for Sirtuins. *Neuron*, *62*(3), 335–348. https://doi.org/10.1016/j.neuron.2009.03.026

Renthal, W., Maze, I., Krishnan, V., Covington III, H. E., Xiao, G., Kumar, A., ... & Nestler, E. J. (2007). Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron*, *56*(3), 517-529. https://doi.org/10.1016/j.neuron.2007.09.032

Roberts, D. C., Corcoran, M. E., & Fibiger, H. C. (1977). On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacology Biochemistry and Behavior*, *6*(6), 615-620. https://doi.org/10.1016/0091-3057(77)90084-3

Rogge, G. A., & Wood, M. A. (2013). The Role of Histone Acetylation in Cocaine-Induced Neural Plasticity and Behavior. *Neuropsychopharmacology*, *38*(1), 94–110. https://doi.org/10.1038/npp.2012.154

Rogge, G. A., Singh, H., Dang, R., & Wood, M. A. (2013). HDAC3 Is a Negative Regulator of Cocaine-Context-Associated Memory Formation. *Journal of Neuroscience*, *33*(15), 6623–6632. https://doi.org/10.1523/jneurosci.4472-12.2013

Romieu, P., Deschatrettes, E., Host, L., Gobaille, S., Sandner, G., & Zwiller, J. (2011). The Inhibition of Histone Deacetylases Reduces the Reinstatement of Cocaine-Seeking Behavior in Rats. *Current Neuropharmacology*, *9*(1), 21–25. https://doi.org/10.2174/157015911795017317

Romieu, P., Host, L., Gobaille, S., Sandner, G., Aunis, D., & Zwiller, J. (2008). Histone Deacetylase Inhibitors Decrease Cocaine But Not Sucrose Self-Administration in Rats. *Journal of Neuroscience*, *28*(38), 9342–9348. https://doi.org/10.1523/jneurosci.0379-08.2008

Ryan, S. A. (2019). Cocaine Use in Adolescents and Young Adults. *Pediatric Clinics*, *66*(6), 1135-1147. https://doi.org/10.1016/j.pcl.2019.08.014

Saunders, J. B., Aasland, O. G., Babor, T. F., De La Fuente, J. R., & Grant, M. (1993). Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption-II. *Addiction*, *88*(6), 791-804. https://doi.org/10.1111/j.1360-0443.1993.tb02093.x

Schroeder, F. A., Lewis, M. C., Fass, D. M., Wagner, F. F., Zhang, Y. L., Hennig, K. M., ... & Petryshen, T. L. (2013). A selective HDAC 1/2 inhibitor modulates chromatin and gene expression in brain and alters mouse behavior in two mood-related tests. *PloS one*, *8*(8), e71323. https://doi.org/10.1371/journal.pone.0071323

Schroeder, F. A., Wang, C., Van de Bittner, G. C., Neelamegam, R., Takakura, W. R., Karunakaran, A., ... & Hooker, J. M. (2014). PET imaging demonstrates histone deacetylase target engagement and clarifies brain penetrance of known and novel small molecule inhibitors in rat. *ACS chemical neuroscience*, *5*(10), 1055-1062. https://doi.org/10.1021/cn500162j

Seo, Y. J., Muench, L., Reid, A., Chen, J., Kang, Y., Hooker, J. M., ... & Kim, S. W. (2013). Radionuclide labeling and evaluation of candidate radioligands for PET imaging of histone deacetylase in the brain. *Bioorganic & medicinal chemistry letters*, *23*(24), 6700-6705. https://doi.org/10.1016/j.bmcl.2013.10.038

Shankman, S. A., Funkhouser, C. J., Klein, D. N., Davila, J., Lerner, D., & Hee, D. (2018). Reliability and validity of severity dimensions of psychopathology assessed using the Structured Clinical Interview for DSM-5 (SCID). *International journal of methods in psychiatric research*, *27*(1), e1590. https://doi.org/10.1002/mpr.1590

Sharma, R. P., Grayson, D. R., & Gavin, D. P. (2008). Histone deactylase 1 expression is increased in the prefrontal cortex of schizophrenia subjects: analysis of the National Brain Databank microarray collection. *Schizophrenia research*, *98*(1-3), 111–117. https://doi.org/10.1016/j.schres.2007.09.020

Shiels, M. S., Freedman, N. D., Thomas, D., & Berrington de Gonzalez, A. (2018). Trends in US drug overdose deaths in non-Hispanic black, Hispanic, and non-Hispanic white persons, 2000–2015. *Annals of internal medicine, 168*(6), 453-455. https://doi.org/10.7326/M17-1812

Sinha, R. (2011). New findings on biological factors predicting addiction relapse vulnerability. *Current psychiatry reports, 13*(5), 398. https://doi.org/10.1007/s11920-011-0224-0

Sled, J. G., Zijdenbos, A. P., & Evans, A. C. (1998). A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE transactions on medical imaging, 17*(1), 87–97. https://doi.org/10.1109/42.668698

Sobell, L. C., Brown, J., Leo, G. I., & Sobell, M. B. (1996). The reliability of the Alcohol Timeline Followback when administered by telephone and by computer. *Drug and alcohol dependence, 42*(1), 49–54. https://doi.org/10.1016/0376-8716(96)01263-x

Sorg, B. A., & Kalivas, P. W. (1991). Behavioral and neurochemical cross-sensitization between footshock stress and cocaine. *Brain Res, 559*, 29-36. https://doi.org/10.1016/0006-8993(91)90283-2.

Spielberger, C. D. (1989). *State-Trait Anxiety Inventory: Bibliography* (2nd ed.). Palo Alto, CA: Consulting Psychologists Press.

Spinhoven, P., Penninx, B. W., Hickendorff, M., van Hemert, A. M., Bernstein, D. P., & Elzinga, B. M. (2014). Childhood Trauma Questionnaire: factor structure, measurement invariance, and validity across emotional disorders. *Psychological assessment, 26*(3), 717. https://doi.org/10.1037/pas0000002

Stewart, J. (2008). Psychological and neural mechanisms of relapse. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*(1507), 3147-3158. https://doi.org/10.1098/rstb.2008.0084

Stewart, J., de Wit, H., & Eikelboom, R. (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychological Review*, *91*(2), 251–268. https://doi.org/10.1037/0033-295x.91.2.251

Sun, H. S., Damez-Werno, D. M., Scobie, K. N., Shao, N.-Y., Dias, C., Rabkin, J., … Nestler, E. J. (2017). Regulation of BAZ1A and nucleosome positioning in the nucleus accumbens in response to cocaine. *Neuroscience*, *353*, 1–6. https://doi.org/10.1016/j.neuroscience.2017.04.007

Sun, J., Wang, L., Jiang, B., Hui, B., Lv, Z., & Ma, L. (2008). The effects of sodium butyrate, an inhibitor of histone deacetylase, on the cocaine- and sucrose-maintained self-administration in rats. *Neuroscience Letters*, *441*(1), 72–76. https://doi.org/10.1016/j.neulet.2008.05.010

Teixeira, C. A. B., Lasiuk, G., Barton, S., Fernandes, M. N. D. F., & Gherardi-Donato, E. C. D. S. (2017). An exploration of addiction in adults experiencing early-life stress: a metasynthesis. *Revista latino-americana de enfermagem*, *25*. http://doi.org/10.1590/1518-8345.2026.2939.

Tesone-Coelho, C., Morel, L. J., Bhatt, J., Estevez, L., Naudon, L., Giros, B., … Daugé, V. (2013). Vulnerability to opiate intake in maternally deprived rats: implication of MeCP2 and of histone acetylation. *Addiction Biology*, *20*(1), 120–131. https://doi.org/10.1111/adb.12084

Torres, O. V., Ladenheim, B., Jayanthi, S., McCoy, M. T., Krasnova, I. N., Vautier, F. A., & Cadet, J. L. (2016). An Acute Methamphetamine Injection Downregulates the Expression of Several Histone Deacetylases (HDACs) in the Mouse Nucleus Accumbens: Potential Regulatory Role of HDAC2 Expression. *Neurotoxicity research*, *30*(1), 32–40. https://doi.org/10.1007/s12640-015-9591-3

Tsankova, N. M., Berton, O., Renthal, W., Kumar, A., Neve, R. L., & Nestler, E. J. (2006). Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nature Neuroscience*, *9*(4), 519–525. https://doi.org/10.1038/nn1659

Tseng, C. E. J., Gilbert, T. M., Catanese, M. C., Hightower, B. G., Peters, A. T., Parmar, A. J., ... & Hooker, J. M. (2020). In vivo human brain expression of histone deacetylases in bipolar disorder. *Translational psychiatry*, *10*(1), 1-9. https://doi.org/10.1038/s41398-020-00911-5

Uchida, S., Hara, K., Kobayashi, A., Otsuki, K., Yamagata, H., Hobara, T., … Watanabe, Y. (2011). Epigenetic Status of Gdnf in the Ventral Striatum Determines Susceptibility and Adaptation to Daily Stressful Events. *Neuron*, *69*(2), 359–372. https://doi.org/10.1016/j.neuron.2010.12.023

Van Dam, N. T., Rando, K., Potenza, M. N., Tuit, K., & Sinha, R. (2014). Childhood maltreatment, altered limbic neurobiology, and substance use relapse severity via trauma-specific reductions in limbic gray matter volume. *JAMA psychiatry*, *71*(8), 917-925. https://doi.org/10.1001/jamapsychiatry.2014.680

Van Velden, F. H., Kloet, R. W., Van Berckel, B. N., Lammertsma, A. A., & Boellaard, R. (2009). Accuracy of 3-dimensional reconstruction algorithms for the high-resolution research tomograph. *Journal of Nuclear Medicine*, *50*(1), 72-80. https://doi.org/10.2967/jnumed.108.052985

Volmar, C.-H., & Wahlestedt, C. (2015). Histone deacetylases (HDACs) and brain function. *Neuroepigenetics*, *1*, 20–27. https://doi.org/10.1016/j.nepig.2014.10.002

Walker, D. M., Cates, H. M., Heller, E. A., & Nestler, E. J. (2015). Regulation of chromatin states by drugs of abuse. *Current Opinion in Neurobiology*, *30*, 112–121. https://doi.org/10.1016/j.conb.2014.11.002

Wang, C., Schroeder, F. A., Wey, H.-Y., Borra, R., Wagner, F. F., Reis, S., … Hooker, J. M. (2014). In Vivo Imaging of Histone Deacetylases (HDACs) in the Central Nervous System and Major Peripheral Organs. *Journal of Medicinal Chemistry*, *57*(19), 7999–8009. https://doi.org/10.1021/jm500872p

Wang, L., Lv, Z., Hu, Z., Sheng, J., Hui, B., Sun, J., & Ma, L. (2010). Chronic Cocaine-Induced H3 Acetylation and Transcriptional Activation of CaMKIIα in the Nucleus Accumbens Is

Critical for Motivation for Drug Reinforcement. *Neuropsychopharmacology*, *35*(4), 913–928. https://doi.org/10.1038/npp.2009.193

Wang, S., Ko, S., Jo, S., Choi, M., Lee, S., Jo, H.-R., … Son, H. (2017). TRPV1 Regulates Stress Responses through HDAC2. *Cell Reports*, *19*(2), 401–412. https://doi.org/10.1016/j.celrep.2017.03.050

Warner, M., Trinidad, J. P., Bastian, B. A., Miniño, A. M., & Hedegaard, H. (2016, December 20). *Drugs most frequently involved in drug overdose deaths: United States, 2010–2014.* https://stacks.cdc.gov/view/cdc/43280/cdc_43280_DS1.pdf

Watt, M. J., Burke, A. R., Renner, K. J., & Forster, G. L. (2009). Adolescent male rats exposed to social defeat exhibit altered anxiety behavior and limbic monoamines as adults. *Behavioral Neuroscience*, *123*(3), 564–576. https://doi.org/10.1037/a0015752

Wey, H.-Y., Gilbert, T. M., Zürcher, N. R., She, A., Bhanot, A., Taillon, B. D., … Hooker, J. M. (2016). Insights into neuroepigenetics through human histone deacetylase PET imaging. *Science Translational Medicine*, *8*(351). https://doi.org/10.1126/scitranslmed.aaf7551

Wey, H.-Y., Wang, C., Schroeder, F. A., Logan, J., Price, J. C., & Hooker, J. M. (2015). Kinetic Analysis and Quantification of [11C]Martinostat for in Vivo HDAC Imaging of the Brain. *ACS Chemical Neuroscience*, *6*(5), 708–715. https://doi.org/10.1021/acschemneuro.5b00066

White, F. J., & Kalivas, P. W. (1998). Neuroadaptations involved in amphetamine and cocaine addiction. *Drug and Alcohol Dependence*, *51*(1-2), 141–153. https://doi.org/10.1016/s0376-8716(98)00072-6

Woicik, P. A., Stewart, S. H., Pihl, R. O., & Conrod, P. J. (2009). The Substance Use Risk Profile Scale: a scale measuring traits linked to reinforcement-specific substance use profiles. *Addictive behaviors*, *34*(12), 1042–1055. https://doi.org/10.1016/j.addbeh.2009.07.001

Wong, D. F., Kuwabara, H., Schretlen, D. J., Bonson, K. R., Zhou, Y., Nandi, A., ... & London, E. D. (2006). Increased occupancy of dopamine receptors in human striatum during cue-

elicited cocaine craving. *Neuropsychopharmacology*, *31*(12), 2716-2727. https://doi.org/10.1038/sj.npp.1301194

Yeh, H. H., Tian, M., Hinz, R., Young, D., Shavrin, A., Mukhapadhyay, U., ... & Gelovani, J. G. (2013). Imaging epigenetic regulation by histone deacetylases in the brain using PET/MRI with 18F-FAHA. *NeuroImage*, *64*, 630-639. https://doi.org/10.1016/j.neuroimage.2012.09.019

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2004). Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *European Journal of Neuroscience*, *19*(1), 181–189. https://doi.org/10.1111/j.1460-9568.2004.03095.x

Yin, H. H., Ostlund, S. B., Knowlton, B. J., & Balleine, B. W. (2005). The role of the dorsomedial striatum in instrumental conditioning. *European Journal of Neuroscience*, *22*(2), 513–523. https://doi.org/10.1111/j.1460-9568.2005.04218.x

Zapata, A., Minney, V. L., & Shippenberg, T. S. (2010). Shift from Goal-Directed to Habitual Cocaine Seeking after Prolonged Experience in Rats. *Journal of Neuroscience*, *30*(46), 15457–15463. https://doi.org/10.1523/jneurosci.4072-10.2010

TABLES AND FIGURES

Table 1. Demographic characteristics, administered radiotracer dose, and behavioural metrics. All values are reported as mean ± standard deviation. *p≤.05 **p≤.001

Metric	Controls (*n*=13)	Cocaine Users (*n*=8)	*p* value
Age (years)	24.4 ± 4.9	24.5 ± 4.0	.750
Sex (female)	9/13	2/8	.080
Body mass index (kg/m²)	22.5 ± 1.5	24.5 ± 3.2	.140
Early life adversity (CTQ score)	32.5 ± 10.8	45.1 ± 20.9	.037*
Injected Dose (mCi)	9.4 ± 1.7	10.2 ± 1.1	.697
Molar activity (mCi/nmol)	1.2 ± 0.8	3.5 ± 2.7	.161
Depressive symptoms (BDI-II score)	2.9 ± 5.0	6.3 ± 6.3	.037*

State anxiety (STAI score)	25.9 ± 7.7	28.9 ± 8.4	.301
Trait anxiety (STAI score)	36.2 ± 6.7	45.6 ± 13.2	.076
Impulsivity (BIS-11 score)	52.6 ± 7.9	66.6 ± 7.5	.001**
Impulsivity (SURPS score)	8.9 ± 1.4	11.4 ± 2.8	.037*
Sensation seeking (SURPS score)	17.9 ± 2.0	19.5 ± 3.0	.140
Introversion/Hopelessness (SURPS score)	9.9 ± 2.8	11.5 ± 2.3	.161
Anxiety sensitivity (SURPS score)	9.5 ± 2.2	11.2 ± 1.8	.121

Table 2. Substance use metrics of study participants. All values are reported as mean ± standard deviation. Fisher's exact test (FET) was used to test for differences in proportion of individuals who ever used the substance, whereas the Mann-Whitney U (MW) test was used to test for group differences in lifetime occasions and current frequency of use. For alcohol, cannabis, cocaine, and amphetamine, current use was calculated as the average number of occasions per month during the past 90 days. For tobacco, current use was measured as cigarettes per day during the past 90 days. *p≤.05 **p≤.001

Substance Use	Controls (*n*=13)	Cocaine Users (*n*=8)	*p* values	
			FET	MW
Alcohol (n, lifetime occasions)	13/13, 408 ± 621	8/8, 1437 ± 1176	-	.008*
Monthly use (n, occ/month)	9/13, 4.78 ± 7.29	8/8, 13.5 ± 11.6	.131	.001**
Alcohol dependence (AUDIT)	2.54 ± 2.85	11.3 ± 5.73		<.001**
Tobacco (n, cigarettes per day)	1/13, 4.00 ± 0.0	5/8, 7.08 ± 3.60	.014*	.037*
Cannabis (n, lifetime occasions)	7/13, 266 ± 484	8/8, 1548 ± 1817	.046*	.001**
Monthly use (n, occ/month)	4/13, 9.88 ± 13.9	7/8, 14.3 ± 14.8	.023*	.016*
Opiates (n, lifetime occasions)	2/13, 6.67.5 ± 5.51	3/8, 19.5 ± 15.4	.325	.114
Cocaine (n, lifetime occasions)	-	8/8, 92.1 ± 75.9		
Time since last use (n, days)	-	8/8, 17.5 ± 14.5		
Monthly use (n, occ/month)	-	8/8, 6.38 ± 9.09		
Amphetamine (n, lifetime occasions)	-	7/8, 46.6 ± 49.6		
Monthly use (n, occ/month)[1]	-	4/8, 1.66 ± 1.06		
MDMA (n, lifetime occasions)	-	8/8, 40.0 ± 44.0		
Ketamine (n, lifetime occasions)	-	4/8, 8.25 ± 9.91		
PCP (n, lifetime occasions)	-	1/8, 3.00 ± 0.0		
Psilocybin (n, lifetime occasions)	-	5/8, 35.5 ± 32.1		
Other hallucinogens (n, lifetime occ.)	-	6/8, 13.9 ± 25.8		
GHB (n, lifetime occasions)	-	3/8, 1.67 ± 1.15		
Salvia (n, lifetime occasions)	-	3/8, 19.7 ± 28.0		
Benzodiazepine (n, lifetime occ.)	-	1/8, 14.0 ± 0.0		
Alkyl nitrite (n, lifetime occasions)	-	2/8, 19.0 ± 12.7		

Figure 1. [^{11}C]Martinostat SUVR in the VST, AST, and SMST. (**A**) Combined ROI mask for the striatum (VST, AST, and SMST) and mean SUVR images for healthy controls (HC, *n*=13) and cocaine users (CU, *n*=8) at MNI coordinates *x*=17, *y*=-4 , *z*=-2. Red arrows indicate the location of the ROIs. (**B**) SUVR extracted from VST, AST, and SMST do not significantly differ between healthy controls (blue) and cocaine users (red). Box plots display median, first quartile, third quartile, minimum, and maximum.

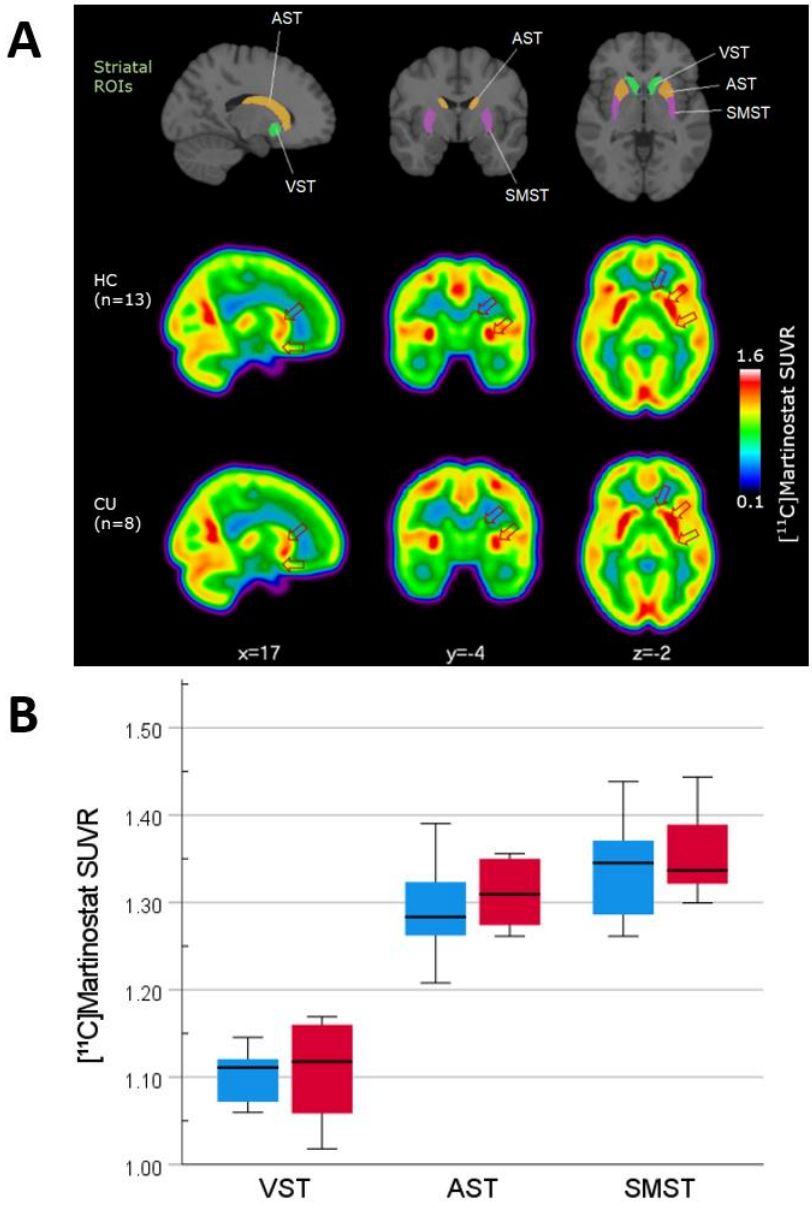

Figure 2. Relationship between current cocaine use and striatal [^{11}C]Martinostat SUVR. Average monthly cocaine use during the past 90 days among cocaine users (*n*=8) significantly predicted [^{11}C]Martinostat SUVR in (**A)** the ventral striatum, (**B**) associative striatum, but not in (**C**) the sensorimotor striatum.

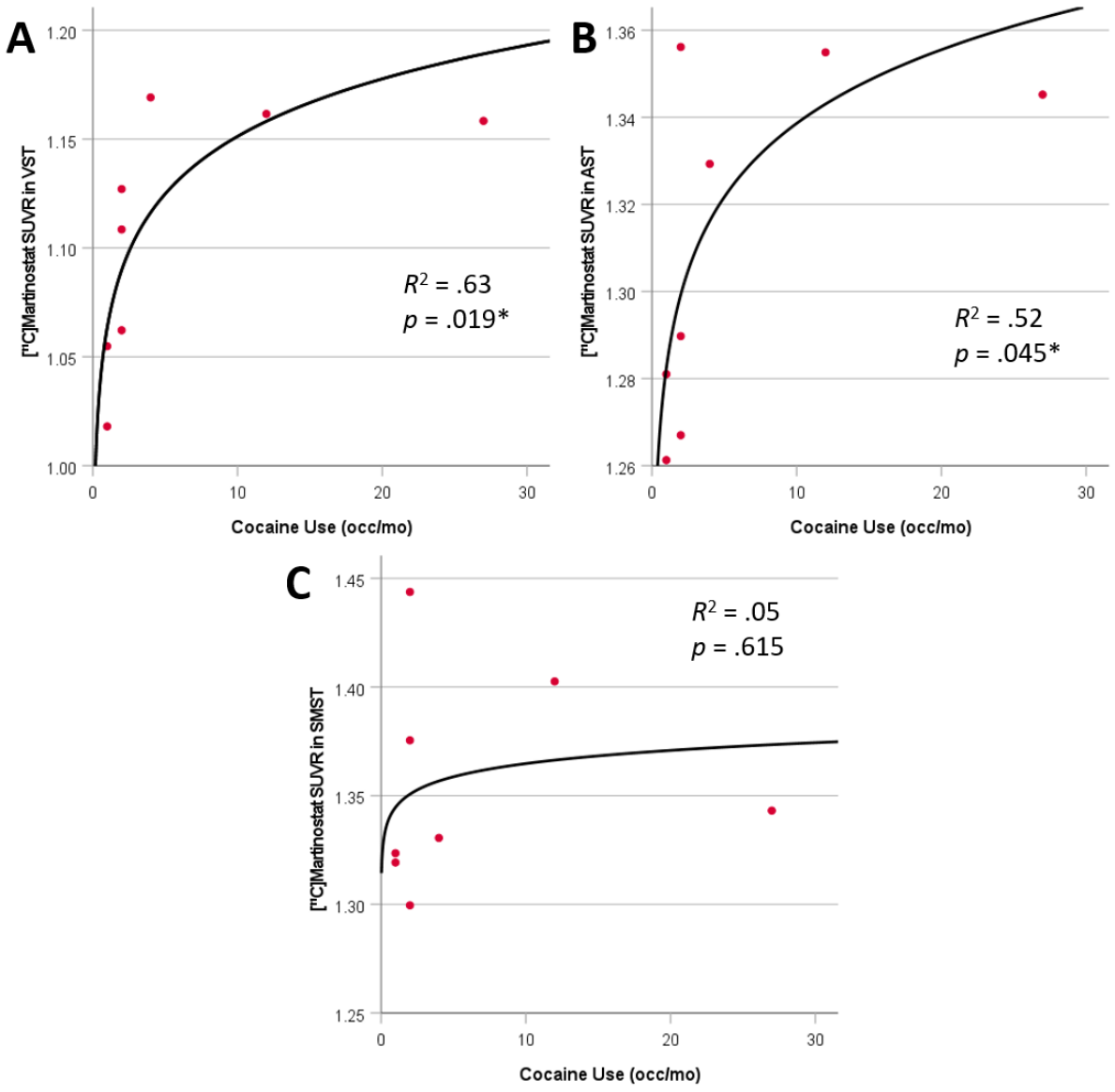

Figure 3. Relationship between duration of abstinence from cocaine and striatal [^{11}C]Martinostat SUVR. Number of days since last cocaine use, among cocaine users (*n*=8), significantly predicted [^{11}C]Martinostat SUVR in (**A)** the ventral striatum, but not in (**B**) the associative striatum or (**C**) sensorimotor striatum

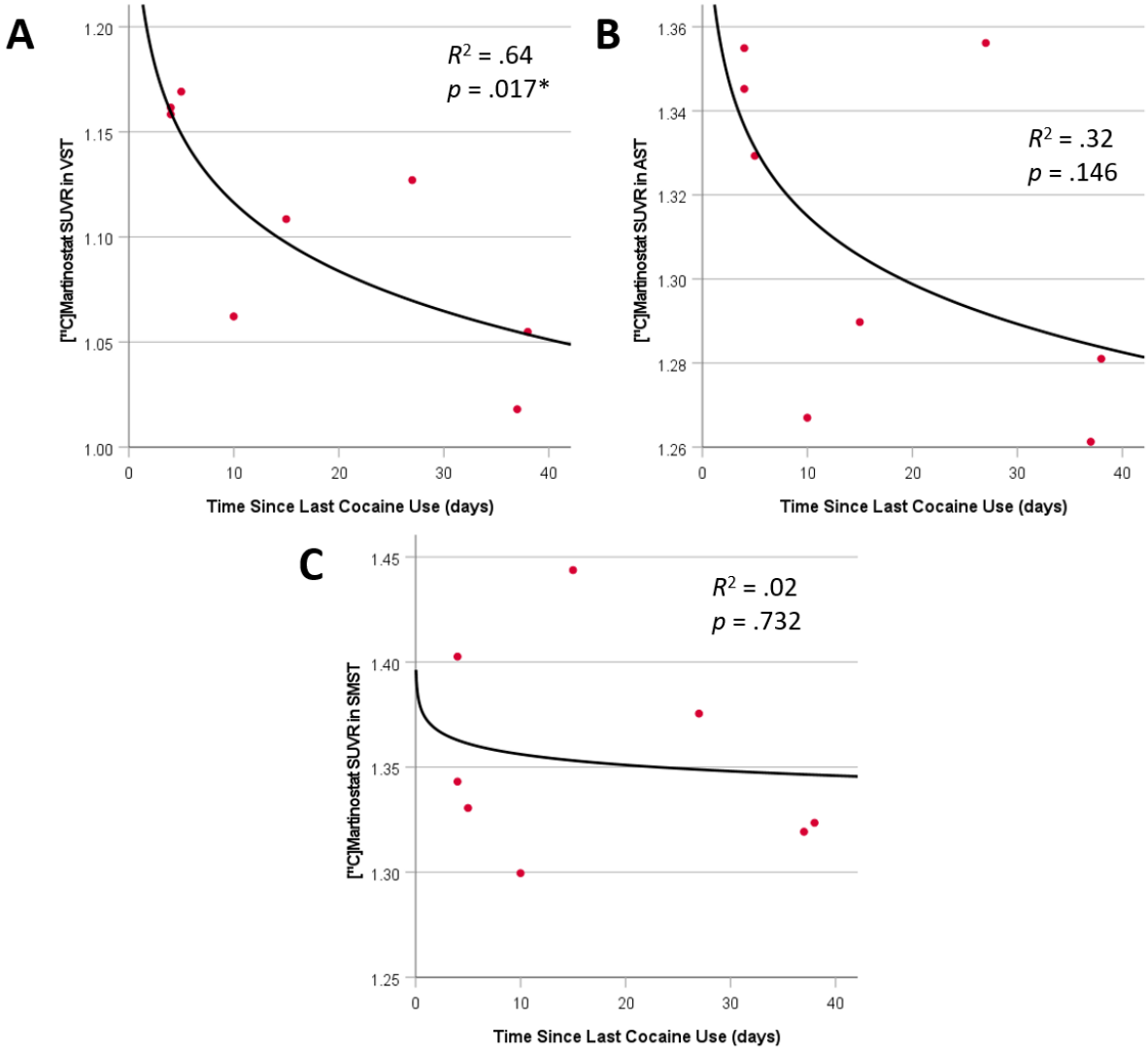

Figure 4. Correlations between striatal [^{11}C]Martinostat SUVR and early life adversity. Total CTQ scores from healthy controls (blue circles, *n*=13) and cocaine users (red circles, *n*=8) were compared with SUVR extracted from (**A**) the ventral striatum, (**B**) associative striatum, and (**C**) sensorimotor striatum, using Spearman rank correlation. *p≤.05