THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOBEHAVIORAL HEALTH

EPIGENETICS AND ADDICTION: A LITERATURE REVIEW

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A thesis
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in Biobehavioral Health and Psychology
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ABSTRACT

Epigenetics can be described as a mechanism for altering gene expression without actually modifying the DNA sequence itself. Epigenetics is a rapidly growing field, as it may be the culprit behind various diseases and health issues. For example, evidence suggests that some part of the development of a drug addiction can be attributed to epigenetic changes in a person’s genome. This literature review examines current research on the relationship between epigenetics and addiction and explores the best methods of studying these mechanisms. Further research on epigenetic mechanisms may uncover key information on how and why individuals develop drug addictions.
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This thesis would not have been possible without the assistance and support I received from countless individuals. I would like to express my sincere thanks to Dr. David Vandenberghe for giving me this opportunity and going above and beyond all of my expectations with his willingness to help me through this process. Dr. Vandenberghe is the perfect example of an academic who is unbelievably passionate about both the pursuit of knowledge and the welfare and success of his students. I could not have asked for a better thesis supervisor.

I would also like to thank Dr. Idan Shalev for being so helpful with his critiques and guidance as the deadline drew near. Finally, I must express my gratitude for every family member, friend, professor, and acquaintance that has helped me learn to believe in myself over these past four years at Penn State. Without the encouragement and support I received from all of these people, this thesis would not have come to fruition.
Chapter 1

Introduction

About 21.5 million people in the United States suffer from some form of drug or alcohol addiction, known collectively as Substance Use Disorder, or SUD (Center for Behavioral Health Statistics and Quality, 2015). SUD can have a tremendous impact on a person’s life, as addicts often struggle to fulfill work and social obligations, spend large amounts of time and money trying to satisfy their cravings, and continue to use substances even when their health is at risk. According to a UN report, approximately 200,000 people worldwide die each year from drug abuse (UN Office on Drugs and Crime [UNODC], 2014). Furthermore, the number of deaths each year from overdose is on the rise, which only highlights the severity of this issue (Center for Behavioral Health Statistics and Quality, 2015).

There are many treatments currently available for people suffering from SUD, including various forms of therapy and medication. The goal of addiction therapy is to identify and prevent thoughts and behaviors that may lead to drug use. Therapy is often paired with medication, which can improve withdrawal symptoms, decrease cravings, and prevent relapse (DrugFacts, 2013). These medications work by targeting the neurotransmitters in the brain that are responsible for addiction.

Different types of drugs have different mechanisms of action, but one common factor among the various types of drug addiction is the role of the mesolimbic system, which is part of the brain’s reward system (DrugFacts, 2013). The reward system is a group of brain structures responsible for the experience of pleasure, and the mesolimbic system refers specifically to the dopaminergic pathway that connects the ventral tegmental area (VTA) to the nucleus accumbens.
Dopamine is the neurotransmitter involved in producing these feelings of pleasure, and drug use increases levels of dopamine in the brain. In this sense, dopamine serves as a reward for drug use, which perpetuates the behavior through a process called reward learning. Reward learning can cause dopamine neurons to prepare to fire in mere anticipation of a rewarding stimulus (Day et al., 2013). The cause of this neural plasticity is unknown, but recent evidence points us towards the area of epigenetics (Bird, 2002; Weinhold, 2006).

Epigenetics is a term used to describe any changes made to an individual’s genome without altering the DNA sequence itself. Epigenetic changes typically result in alterations in gene expression and can occur through a variety of mechanisms, including chromatin modification and DNA methylation (Weinhold, 2006). Chromatin is the structure that stores a cell’s DNA, bound tightly around proteins called histones. The epigenetic process of chromatin modification involves the structural alteration of chromatin, which can prevent transcriptional factors from accessing the DNA, thereby altering gene expression (Weinhold, 2006). On the other hand, DNA methylation, which is the epigenetic mechanism that will be the primary focus in this review, involves the addition of a methyl group to the DNA. Methylation most commonly occurs on the nucleotide cytosine at sites along the DNA where the cytosine is followed by a guanine, also called a CpG site (Jones & Takai, 2001). Rather than being distributed evenly along the genome, CpG sites are found in clusters called CpG islands (Robison & Nestler, 2011). Prior research conducted on embryonic stem cells has shown that DNA methylation can occur at other locations (Pinney, 2015), but this review will specifically focus on methylation at CpG sites.

The alterations in the brain that contribute to addiction are thought to be caused by epigenetic changes that occur in the mesolimbic system as a result of using drugs (Nestler,
The purpose of this review is to examine the existing research on the role that epigenetics plays in drug addiction with the intention of identifying appropriate directions for future research. I specifically aimed to gather research on the relationship between DNA methylation and addiction, the concept of epigenetic inheritance, and the comparability of methylation in buccal cells to methylation in brain cells.
Chapter 2

Methods

This literature review includes 22 peer-reviewed research articles, primarily collected from the online databases PubMed and ProQuest. Figure 1 depicts the keywords that I used to retrieve these articles and shows how many articles I pulled from each database. Out of the research papers that my searches yielded, I selected only the papers that were relevant to my three main research goals. I identified relevant articles by scanning through the titles and reading the abstracts of papers whose titles indicated a focus on epigenetics and addiction, transgenerational epigenetics, or buccal cell and brain cell research. By reading the abstracts, I determined which articles actually provided valuable information for my analysis and ultimately selected 22 articles.
Figure 1. Literature Search Summary

- 4 articles providing background information on epigenetics
  - ProQuest Search: “epigenetics” AND “addiction”
    1,382 articles retrieved, 4 articles selected

- 7 articles about methylation and addiction
  - PubMed Search: “DNA methylation” AND “addiction”
    131 articles retrieved, 1 article selected
  - ProQuest Search: “DNA methylation” AND “addiction”
    703 articles retrieved, 2 articles selected
  - 4 articles located through other sources

- 6 articles about transgenerational epigenetics
  - PubMed Search: “transgenerational” AND “epigenetics”
    289 articles retrieved, 2 articles selected
  - ProQuest Search: “transgenerational” AND “epigenetics”
    886 articles retrieved, 3 articles selected
  - 1 article located through other sources

- 5 articles about buccal cell DNA methylation
  - PubMed Search: “Buccal cells” AND “DNA methylation”
    79 articles retrieved, 1 article selected
  - ProQuest Search: “Buccal cells” AND “DNA methylation” AND “surrogate”
    1,660 articles retrieved, 3 articles selected
  - 1 article located through other sources
Chapter 3

DNA Methylation and Addiction

While research points to a link between DNA methylation and drug addiction, the precise details surrounding the relationship are still unknown. Day and colleagues (2013) set out to test the hypothesis that DNA methylation and reward-learning are linked and then search for further information about how this relationship functions. Day et al. conditioned a group of Sprague-Dawley rats to expect a sucrose pellet every time that they heard a ten-second tone if they poked their nose into a hole in the cage. Another group of rats was exposed to the same tones, but the sucrose was delivered sporadically so that the rats would not associate the tone with the reward. A third group of rats was repeatedly exposed to the tone and no reward. Seven days after the procedure, the rats were exposed to the same conditions as before. The rats that received the pellet consistently after the tone during the first part of the experiment demonstrated more “nose-pokes” during the tone in phase two than the two other groups of rats, indicating that they had learned to expect the sucrose reward after the tone. Day et al. (2013) then extracted DNA from the VTA and NAc and analyzed the methylation patterns.

The researchers found that hypermethylation occurred in the VTA of the conditioned rats, while there were no differences in methylation identified in the NAc of the experimental and control groups (Day et al., 2013). To further test the role of methylation in reward-learning, Day and colleagues repeated the experiment and injected a subset of the rats with RG108, which inhibits methylation, before the conditioning sessions. The rats injected with RG108 did not display any extra nose-pokes during the tone in the second part of the experiment, which indicates that reward learning did not occur for them as it should have. Further examination of the rats’ brains showed no change in methylation patterns in the conditioned group. This study
demonstrates that DNA methylation is a crucial part of natural reward learning and that this kind of learning may not occur without it. These findings imply that DNA methylation may play an important role in the development of a drug addiction, which results from reward-learning (Day et al., 2013). However, this study only looked at natural reward learning, as drugs were not involved in the experiment. A later study conducted by Massart and colleagues (2015) specifically examined the relationship between DNA methylation and cocaine craving.

In Massart et al.’s study, the experimental group of rats were given the chance to self-administer cocaine so that they would become addicted. Some of these rats were then subjected to a 30-day withdrawal period, while the rest were examined after one day of withdrawal. At the end of the withdrawal period, researchers determined that the rats that were addicted to the cocaine had more methylated CpG sites in their NAcs than the rats in the control group. Since hypermethylation appeared to be associated with addiction, Massart et al. decided to repeat the study and inject a methylation inhibitor, RG108, into the brains of the rats receiving the cocaine. As they expected, the rats that were injected with RG108 exhibited less cocaine seeking during the 30 day withdrawal period than those that did not receive an injection of RG108. Massart and his colleagues even repeated the experiment with sucrose pellets instead of cocaine and still found that the rats that were given the methylation inhibitor pressed the administration lever significantly less than the rats that weren’t (Massart 2015). The fact that inhibiting methylation appears to be able to prevent addiction has incredible implications for the development of drug addiction treatments and prevention.

When considering whether or not this process of inhibiting DNA methylation is applicable to humans, researchers may wonder if this inhibition is permanent or if it could have adverse effects on learning and memory. To test the permanence of inhibiting methylation,
Miller and Sweatt used fear conditioning on rats before and after injecting them with a DNA methyltransferase inhibitor (2007). Rats were either assigned to the control group, which received the fear conditioning and no injection, or the experimental group, which received the injection before fear conditioning. 24 hours later, both groups of rats were brought back into the lab and tested to see how fearful they were. The rats that were not injected with the methylation inhibitor were much more fearful and demonstrated more freezing behaviors than the rats in the experimental group. Both groups then were subjected to fear conditioning once again. This time, neither group received the inhibitor. The control group still demonstrated more fearful behaviors, but the experimental group, which had been injected with the methylation inhibitor two days prior, showed the same amount of fearful behaviors as the control group did on the first test day. This finding that methylation is connected to fearful behavior is important because it shows that inhibiting methylation did not cause permanent damage to their brains, which means it could be an avenue for future research regarding treatments. Taken together, these studies suggest that DNA methylation might play an important role in the behaviors related to addiction in a manner that does not alter other behaviors.
Chapter 4

Transgenerational Epigenetics

We know that drug use causes epigenetic changes to an individual’s DNA, but are those epigenetic changes heritable? If epigenetic changes can, in fact, be passed on through generations, this will direct researchers towards the exact mechanisms of heritability for not only drug addiction but perhaps other health issues as well. Identification of these processes may lead to groundbreaking conclusions about how we can prevent certain health conditions, starting at or even before birth. Emerging research on transgenerational epigenetics is certainly promising.

Numerous research teams have studied the heritability of epigenetic changes in relation to conditions other than drug addiction. For example, a study conducted by Dias and Ressler (2014) demonstrated that olfactory fear conditioning in male rats can increase odor sensitivity in the following four generations of offspring. These results occurred even when the rats were bred using in-vitro fertilization and the male rats were kept completely separated from their mates and offspring, which ensures that the increased sensitivity to the specific odor is a result of epigenetic mechanisms instead of behavioral learning (Dias & Ressler, 2014). Another phenotype that may be passed on to offspring through epigenetics is stress and depression.

Weaver et al. (2004) first discovered that maternal grooming was associated with epigenetic changes in rat pups. Pups whose mothers exhibited a lot of licking, grooming, and arched-back nursing had differences in DNA methylation, which persisted into adulthood. These rats also showed more modest stress responses in adulthood compared to rats that experienced lower amounts of grooming as pups. These results indicate that maternal care, epigenetic changes, and offspring stress responses are all linked (Weaver et al., 2004). Dietz and colleagues
chose to look more closely at the link between epigenetics and the transgenerational inheritance of stress while controlling for environmental differences.

Dietz et al. (2011) designed a study in which they exposed a group of male mice to stress before breeding them. The male mice were removed once the females became pregnant, and the offspring were exposed to a social defeat paradigm after about 8 weeks. The offspring of the stressed mice were significantly more likely to exhibit social avoidance after being exposed to this defeat condition than the offspring in the control group, whose fathers were not subjected to stress before mating (Dietz et al., 2011). This evidence that epigenetic traits can be passed down to offspring without parenting or environmental influences has sparked research on epigenetic inheritance of drug and alcohol addiction.

Maternal exposure to cocaine has been shown to alter global DNA methylation in offspring, which indicates that drug-driven epigenetic changes can be passed down to offspring (Novikova et al., 2008). Evidence also suggests that paternal exposure to cocaine can have epigenetically-caused phenotypic effects on offspring (Vassoler, White, Schmidt, Sadri-Vakili, & Pierce, 2013). In a study using Sprague-Dawley rats, the offspring of cocaine-exposed sires self-administered less cocaine than the offspring of cocaine-naïve sires. The same self-administration test was completed by the offspring using sucrose pellets instead of cocaine, and there was not a significant difference in self-administration between groups, demonstrating that the differences in cocaine administration were not due to learning deficits (Vassoler et al., 2013). These results oddly contradict the idea that the children of drug users are more likely to be users themselves, and a study on parental alcohol exposure had surprisingly similar results.

Finegersh and Homanics (2014) exposed male rats to ethanol vapor for eight hours each day for five weeks, which is the length of the rats’ spermatogenesis cycle. Those male rats were
then placed in cages with female rats and removed after they mated. At the age of eight weeks, the offspring of those rats went through a variety of tests so that researchers could see how they reacted to alcohol. Like the rats in Vassoler et al.’s study (2013), the offspring of ethanol-exposed fathers drank significantly less alcohol than the control group when offered the choice between water and alcohol (Finegersh & Homancis, 2014). These links between adult and offspring cocaine and alcohol consumption offer valuable evidence of epigenetic inheritance regarding drug use and demonstrate that epigenetic changes may not lead to increased drug use as one might speculate.
Chapter 5

Substitutes for Studying Neural Cells

It is important to consider the resources available to researchers when proposing future studies. One of the biggest challenges in studying the link between epigenetics and addiction in humans is the fact that it is not currently possible to study DNA methylation in a living human brain. To combat this problem, many scientists have searched for proxy tissues that are easier to access. Some non-invasive proxies for inaccessible tissues are buccal (cheek) cells, saliva cells, and white blood cells (Smith et al., 2014). Up until recently, blood has been the most commonly used surrogate tissue when studying DNA methylation, but there was not much evidence that blood cells are actually a better alternative than buccal cells (Lowe et al., 2013). In fact, blood cells originate from a different germ layer than brain cells, while buccal cells are from the same layer as brain cells (Farre et al., 2015). Another advantage of using buccal cells is that they are not nearly as diverse as white blood cells are, since blood cells can be categorized into subtypes, while buccal cells are homogenous (Lowe et al., 2013). Lowe and his team decided to search for a scientific basis for using one type of cell over the other. They used whole-genome bisulfite sequencing to compare methylation in the blood to methylation in buccal cells and then identify similarities between those two surrogates and stem, brain, kidney, liver, and sperm cells.

Their study found that buccal cells contained more hypomethylated regions than blood cells, which may indicate more variability in methylation patterns, making buccal cells the better choice for a surrogate tissue. Furthermore, compared to blood cells, the clusters of CpG sites found in buccal cells were more similar to those found in each of the other tissues examined (Lowe, 2013). However, some phenotypes are still more closely associated with variation in methylation patterns in blood cells. For example, hypomethylated regions in blood cells are
associated with autoimmune diseases, such as celiac and Graves’ disease. Hypomethylated regions in buccal cells, on the other hand, are related to disorders of epithelial cells, such as cancer (Lowe, 2013). Since the best surrogate tissue varies depending on the phenotype being studied, Lowe and colleagues were unable to make one generalized recommendation for researchers choosing surrogate tissue.

Two years later, Jiang et al. also conducted a study to determine if DNA methylation patterns in blood cells and buccal cells are congruent (2015). They were able to support Lowe’s findings that DNA methylation is definitely tissue-specific and occurs more in peripheral blood mononuclear cells than buccal cells (Jiang et al., 2015). They also found that methylation in buccal cells varied more across phenotypes, which might make them appear to be superior to study; however, the phenotype that was highlighted in this study was BMI, which turned out to be more closely related to blood cell methylation than buccal cell methylation (Jiang et al., 2015). The overall conclusion drawn from the studies conducted by Lowe, Jiang, and colleagues is that a significant aspect of DNA methylation is tissue-specific, and the phenotype in question is what should determine which type of surrogate tissue to use. Although both studies identified similarities in methylation patterns between buccal cells and brain cells, there were also many differences, and there is no clear evidence to suggest that buccal cells can accurately serve as surrogates for brain cells in DNA methylation studies.
Chapter 6

Discussion

Drug addiction and substance abuse are huge problem around the world, and although there are therapies and medications that can help someone overcome addiction, millions of people still struggle to quit abusing drugs. Epigenetics research could change that. Multiple studies have shown that blocking DNA methylation in the parts of the brain associated with rewards and memory can prevent addiction in rats and mice. Future research should focus on addiction and DNA methylation in humans. Because the literature is inconclusive on whether or not buccal cells are an appropriate surrogate for brain cells when studying methylation and addiction, future studies should first focus on answering that question. Careful comparisons between buccal and brain cell methylation patterns in multiple animal models of addiction might reveal whether buccal cells can serve as proxies for the brain cells that are largely inaccessible.
BIBLIOGRAPHY


# ACADEMIC VITA

## Lauren E. Doberstein
laurendoberstein25@gmail.com

## Education

<table>
<thead>
<tr>
<th><strong>The Pennsylvania State University, Schreyer Honors College</strong></th>
<th><strong>University Park, PA</strong></th>
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<tbody>
<tr>
<td><strong>College of Health and Human Development</strong></td>
<td><strong>Class of 2016</strong></td>
</tr>
<tr>
<td>Bachelor of Science, Biobehavioral Health</td>
<td></td>
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<tr>
<td>Bachelor of Science, Psychology</td>
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## Work Experience

<table>
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<tr>
<th><strong>Leadership and Innovation Lab</strong></th>
<th><strong>University Park, PA</strong></th>
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<tbody>
<tr>
<td><strong>Undergraduate Research Assistant</strong></td>
<td><strong>July 2015-Present</strong></td>
</tr>
<tr>
<td>- Collaborate with team members to develop lab studies and support dissertation project research</td>
<td></td>
</tr>
<tr>
<td>- Analyze errors of famous leaders from historical biographies</td>
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<tr>
<th><strong>Penn State Residence Life</strong></th>
<th><strong>University Park, PA</strong></th>
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<tr>
<td><strong>Resident Assistant</strong></td>
<td><strong>June 2014-May 2015</strong></td>
</tr>
<tr>
<td>- Supervised a floor of 50 diverse students and enforced residence hall policies</td>
<td></td>
</tr>
<tr>
<td>- Developed and utilized crisis management and conflict resolution skills</td>
<td></td>
</tr>
<tr>
<td>- Planned monthly community building events for a residence hall of over 400 students</td>
<td></td>
</tr>
<tr>
<td>- Implemented educational programming for the Schreyer Honors College Special Living Option</td>
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## Activities

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<tr>
<th><strong>Mortar Board Honor Society, Archousai Chapter</strong></th>
<th><strong>University Park, PA</strong></th>
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<tbody>
<tr>
<td><strong>Director of Public Relations</strong></td>
<td><strong>April 2015-April 2016</strong></td>
</tr>
<tr>
<td>- Maintained the organization’s website and social media accounts</td>
<td></td>
</tr>
<tr>
<td>- Played a crucial role in the recruitment, selection, and initiation of the next class</td>
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<tr>
<th><strong>Penn State Dance Marathon</strong></th>
<th><strong>University Park, PA</strong></th>
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<tr>
<td><strong>Dancer Relations Committee Member</strong></td>
<td><strong>October 2015-Present</strong></td>
</tr>
<tr>
<td>- Provide physical and emotional support to THON “dancers” during the 46 hour marathon</td>
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<tr>
<th><strong>Springfield Public Relations Chair</strong></th>
<th><strong>March 2014-March 2015</strong></th>
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<tr>
<td>- Served on a team of 10 executive board members of Springfield – an independent, student-run organization that raised $270,000 for pediatric cancer research in 2015</td>
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<tr>
<td>- Managed social media accounts and designed the organization’s new website</td>
<td></td>
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<tr>
<td>- Led recruitment efforts and brought over 100 new students into the organization, increasing the membership size by about 75% from the previous year</td>
<td></td>
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<tr>
<td>- Presented at weekly meetings of more than 200 general members</td>
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</table>
Springfield Community Chair  
March 2013-March 2014
- Created team unity by planning social and community building events that brought the organization together
- Revamped the social structure of the organization by selecting and managing 10 “Social Captains”

Volunteer Experience

Penn State LifeLink Student Mentor  
January 2016-Present
- Tutored a student in from Special Education program at State College Area High School in Language Arts once weekly
- Dedicated approximately 2 hours each week to eating and socializing with students in the program to help them develop social skills and become acclimated into the Penn State community

Penn State Welcome Week Captain  
August 2015
- Directed a committee of volunteers in the task of helping new students move into their residence halls

Mid-State Literacy Council Tutor  
January 2015-May 2015
- Taught a beginner course in English as a Second Language to adult learners for four hours each week

Relay For Life Participant  
2010-2016
- Fundraised for the American Cancer Society and participated in the annual relay walk; served as a team captain for Relay For Life 2016