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Chronic cocaine use and its epigenetic role in altering proteins involved in memory

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ABSTRACT

Chronic exposure to cocaine results in long-term changes in brain structure and function. Long-term changes are also seen on a microscopic level in terms of alterations in chromatin remodeling and gene expression. These cellular changes result in behavioral changes and can be passed onto the next generation. Heritable changes in gene expression is known as epigenetics and are due to modifications that alter chromatin structure allowing for changes in accessibility of a gene (Browne, Godino, Salery & Nestler, 2020). Despite advances in epigenetic research involving addiction, the mechanisms that underlie the switch from recreational use to addiction is still under much investigation. While environmental factors that put an individual at risk for drug addiction are just as important as genetic factors, a better understanding of how epigenetics plays a role may lead to better psychopharmacological treatment options and decrease the stigma that addiction is due to an individual's lack of morals or willpower if it is framed in the context of being a brain disorder. This review will explore the genes responsible for FosB/ΔFosB and G9a. These proteins are altered following cocaine use and play a role in augmenting drug-related memories. This augmentation in drug-related memories may increase the risk of relapse in an individual, which is a major hurdle in overcoming drug addiction. In addition, this review will explore potential treatments in drug addiction to reduce the health and social costs associated with this disease.

BACKGROUND

Drug Addiction

According to the National Institute of Drug Abuse report in 2017, 19.7 million Americans suffer from drug addiction (NIDA, 2017). Addiction is characterized by the inability to abstain from a drug despite previous attempts to do so and impacting your daily life in a negative way. Addiction may be associated with dependence. Physical dependence includes tolerance and withdrawal. Tolerance is having to take a higher dose of the drug after each subsequent use to achieve the same effect (American Addiction Centers, 2020). Withdrawal symptoms are negative physiological symptoms caused by cessation of a drug after chronic use (American Psychiatric Association, 2013). Withdrawal symptoms are different for every drug, but cocaine withdrawal is characterized by agitation, restlessness, anxiety, fatigue, increased appetite, depressed mood and suicidal thoughts. Psychological dependence includes obsession with obtaining and taking a substance as well as cravings. Relapse, returning to a substance after previous attempts to remain abstinent, is a common setback in the recovery of addiction. The risk of relapse may last a lifetime and can be triggered by places, people or things associated with drug use (American Addiction Centers, 2020). Repeated cocaine use promotes neural plasticity in brain regions involved in the reward pathway, leading to drug addiction (Luján, Castro-Zavala, Alegre-Zurano & Valverde, 2018).

The reward pathway

Repeated exposure to cocaine results in changes in the brain regions associated with the dopaminergic reward circuitry. These areas include but are not limited to the nucleus accumbens (NAc), ventral tegmental area (VTA), prefrontal cortex (PFC), the hippocampus, and the amygdala (Browne et al., 2020). This is known as the mesolimbic pathway which is involved in the reward pathway. The reward pathway builds motivation for survival which may include

object, stimuli or activity that have a positive value. Evolutionarily, it is important in reinforcing activities involved in reproduction and survival such as seeking sex and food. This pathway includes the VTA, which is responsible for motivation, and the NAc which is involved in the reinforcing effects of cocaine. Dopaminergic pathways from the VTA to NAc and from the VTA to the mPFC are central to reward circuitry (Hamilton & Nestler, 2019). Cocaine produces a buildup of dopamine in these regions. The amount of dopamine produced in the NAc due to cocaine exposure exceeds the natural amounts of dopamine produced during everyday activities, which produces the pleasurable effect associated with taking cocaine. Limbic structures such as the hippocampus and amygdala are important in this pathway as well. The hippocampus is involved in memory which helps us remember how we obtained a reward. The amygdala is thought to play a role in consolidation of the emotionally charged stimulus associated with a reward. In regard to addiction, these structures help us associate a drug with people, places and things. These drug cues may trigger cravings or relapse. Glutaminergic neurons from the PFC to the VTA act as a regulatory pathway. The PFC integrates information and is involved with self-control. This pathway may be impaired in individuals struggling with addiction, making it harder to resist the cravings of a drug. GABAergic neurons from the NAc to the VTA are also involved in inhibiting the dopaminergic pathway and may also be impaired in addiction (Nestler, 2005).

Epigenetics

Epigenetics describes changes in DNA expression, but not changes in the DNA sequence itself. DNA wrapped tightly around proteins called histones make up chromatin. Chromatin is densely packed which allows for it to fit inside of the nucleus of a cell. There are two forms of chromatin. Euchromatin is loosely wound around histones which allows the transcriptional machinery easier access to DNA. This allows for DNA to be duplicated, undergo transcription to

become mRNA, and finally undergo translation to form protein. Heterochromatin, on the other hand, is tightly wound which gives the transcriptional machinery less access to the DNA, resulting in repression of a given gene. Post-translational modifications (PTMs) which as its name suggests, happens after translation has occurred, is a type of reversible epigenetic regulatory effect. The majority of PTMs occur on histones because they protrude outwards and are the most accessible. PTMs alter chromatin accessibility which lead to changes in gene expression. There are many types of PTMs, but acetylation and methylation are the most abundant PTMs involved in cocaine exposure. In general, acetylation is associated with euchromatin while methylation is associated with heterochromatin. However, it is important to note that there are some exceptions to this rule. Histone acetyltransferases (HATs) acetylate histones while histone deacetylases (HDACs) take the acetyl group off histones. Methylation is catalyzed by DNA methyltransferases (DNMTs). PTMs are put on histones via writer enzymes, are interpreted via reader proteins and can be removed via eraser enzymes. It is a combination of these writer, reader and eraser proteins that regulate the expression of a given gene (Hamilton & Nestler, 2019).

Repeated cocaine use results in differing expressions of proteins involved in the reward pathway which may perpetuate drug-related behaviors leading to addiction. While various brain areas are altered following chronic cocaine-use, this review will explore the mechanisms in which brain regions responsible for memory are altered. The proteins altered following chronic cocaine-use are FosB/ΔFosB (Hamilton & Nestler, 2019) and G9a (Maze et al., 2010). The increase of these proteins following cocaine-use are altered through epigenetic mechanisms and enhance the memory of drug-associated cues. Pharmacological treatments that impair memory of

drug-associated cues in addicted individuals may be promising treatments in helping to overcome relapse.

PROTEINS IVOLVED IN MEMORY SUBJECTED TO EPIGENETIC REGULATION FOLLOWING COCAINE USE

ΔFosB upregulation in the hippocampus is important for cocaine-related learning

ΔFosB is a splice variant of FosB and is a transcription factor that plays a role in addiction. ΔFosB is harder to degrade and has a longer half-life than FosB (Gajweski et al., 2019). While ΔFosB is normally present in small quantities in the NAc and other brain regions involved in addiction, chronic exposure to cocaine results in an increase of ΔFosB. Overtime, ΔFosB accumulates and remains increased even after weeks of not taking cocaine. Mice with elevated ΔFosB exhibit behaviors similar to addictive behaviors seen in humans (Nestler, 2005). One study found that ΔFosB alters hippocampal synapses and this has an effect on spatial learning (Eagle et al., 2015). Gajewski et al. wanted to examine how chronic cocaine exposure induces changes of ΔFosB, specifically in the hippocampus (2019). In addition, they also wanted to examine what epigenetic modifications are responsible for this induction. The researchers exposed mice to cocaine (20mg/kg) for 30 minutes a day for 10 days via an intraperitoneal injection. They also had a control group that was given saline. They found that mice given cocaine had an increase in FosB and ΔFosB in the ventral and dorsal hippocampus. More specifically, there was an increase in the number of neurons expressing FosB and ΔFosB in the CA1 region of both the ventral and dorsal hippocampus (Gajewski et al., 2010).

To see whether this increase in FosB isoforms had an impact on memory, researchers used a viral vector, adeno-associated virus (AAV) to express green fluorescent protein (GFP) and ΔJunD. GFP allows you to see a specific protein, while ΔJunD binds to ΔFosB to silence it. As a control, the used AAV-GFP to look at the amount of ΔFosB protein. To test memory, a novel

object recognition paradigm was used. Mice were exposed to two identical objects in a training-arena. Twenty-four hours later, mice were put back into the arena where one of the objects was replaced with a different object. If mice were to display long-term memory, you would expect them to explore the new object since rodents have an innate curiosity for novel things. Mice expressing AAV-ΔJunD showed impairment in memory as observed by their equal preference for both objects. In comparison, mice expressing AAV-GFP showed more preference for the novel object, indicating intact memory. In addition, researchers tested conditioned place preference on these mice. Conditioned place preference observes the association and preference a rodent makes to a specific area where a positive stimulus was introduced, in this case, cocaine. This indirectly tests drug efficacy (Bardo & Bevins, 2000). They found that mice expressing AAV-ΔJun had decreased conditioned place preference, while AAV-GFP mice had increased place preference. Next, researchers used chromatin immunoprecipitation (ChIP) to see if the increase in ΔFosB in cocaine exposed mice was due to an epigenetic modification (Gajewski et al., 2010). They looked at H3K9me2 since this epigenetic modification has been shown to be responsible for an increase in ΔFosB in the NAc (Damez-Werno et al., 2012). They found a decrease in H3K9me2 at multiple sites along the FosB promoter (Gajewski et al., 2010). Taken together, these results suggest that chronic cocaine exposure leads to an increase in FosB and ΔFosB in the ventral and dorsal regions of CA1. This increase is important for memory including spatial memory, which could contribute to perpetuate drug-seeking behaviors and relapse. In addition, results suggest that a decrease of H3K9me2 at the FosB promoter is responsible for the increase in ΔFosB seen in mice exposed to cocaine.

Reduction of G9a activity in the NAc increases conditioned placed preference

Repeated exposure to cocaine has shown to decrease H3K9 methylation at specific gene promoters in the NAc (Renthal et al., 2009). Maze et al. found that G9a and G9a-like protein (GLP) are two enzymes that catalyze the methylation at H3K9 (2010). They wanted to know the significance of G9a repression after repeated exposure to cocaine. Since ΔFosB has been shown to be increased in the NAc following cocaine exposure, they wanted to see if ΔFosB induction influenced H3K9 methylation and G9a expression. They used mice bred to have increased ΔFosB and found reduced H3K9 methylation as well as reduced G9a expression in the NAc. In support of this finding, mice injected with AAV-ΔFosB showed decreased levels of G9a in the NAc. To further assess the role of G9a, mice were injected with herpes simplex virus (HSV) vector expressing G9a in the NAc to overexpress this protein. GFP was used as a control. They observed conditioned place preference and found that HSV-G9a mice had a decreased preference for cocaine as compared to controls. In addition, mice who were injected with AAV-Cre to knockdown G9a in the NAc showed increased conditioned place preference for cocaine (Maze et al., 2010).

Researchers also wanted to look at the role of G9a in dendritic spine density in the NAc since chronic cocaine use has been shown to increase dendritic spine density in the medium spiny neurons in the NAc. They found that following repeated exposure to cocaine, there was decreased G9a binding and decreased methylation at H3K9 at gene promoters involved in dendritic spine plasticity, leading to an increase in dendritic spine density. Next, they injected mice with either HSV-GFP or HSV-G9a following repeated cocaine exposure to see if the increase in dendritic spine density could be blocked by overexpression of G9a. Overexpression of G9a alone was not enough to decrease dendritic spine density, so this effect must be mediated

by ΔFosB. To examine the interactions between ΔFosB and G9a, they used transgenic mice overexpressing ΔFosB that showed decreased G9a and H3K9me2. They found decreased G9a binding at genes involved in dendritic spine plasticity leading to increased density. Further, they found that AAV-ΔJunD mice blocked increase spine density following repeated cocaine exposure. They also found that in response to chronic cocaine exposure, G9a expression was suppressed and G9a binding to the FosB gene was decreased. As a result, these mice showed increased NAc spine density. In response to acute cocaine administration, G9a levels and its binding to the FosB gene increase. This data suggests that this works as an autoregulatory loop where G9a limits induction of ΔFosB at first, but as ΔFosB accumulates with chronic cocaine use, G9a is repressed (Maze et al., 2010). Taken together, these results show that chronic cocaine use results in an increase in ΔFosB in the NAc which is responsible for decreasing the activity of G9a, thus decreasing H3K9 methylation in this region. In addition, decreased G9a binding to the FosB gene after cocaine use promotes cocaine preference through genes known to regulate dendritic plasticity. However, overexpression of G9a alone is not enough to decrease dendritic spine density in the NAc and must be mediated by ΔFosB. Furthermore, the difference in G9a activity in response to acute cocaine administration versus chronic may further illustrate the molecular switch from drug abuse to addiction.

POTENTIAL TREATMENT OPTIONS

The importance of treating drug addiction

It is no doubt that the opioid epidemic has been a significant contributor to overdose deaths since its increased prescription for pain management in the 1990's (CDC, 2020). In 2017, the opioid epidemic was declared a public health emergency. Cocaine use disorder is a significant risk factor for opioid use disorder. If taken together, these drugs increase the risk of respiratory depression leading to death. The number of deaths involving cocaine has been on the rise since 1999 and rose 60% after 2010 (McCall Jones, Baldwin & Compton, 2017). This increase in overdose deaths corresponds with the second wave of the opioid epidemic, when heroin and synthetic opioid use were on the rise and there was an increase in illicit fentanyl production (CDC, 2020). Data indicates that individuals using cocaine alongside fentanyl are especially at high risk for overdose due to opioid-induced respiratory depression (Klar et al., 2016).

Drug abuse and addiction put an individual at risk for a wide range of both mental and physical health problems. Cocaine use puts an individual at risk for issues such as STDs, HIV, cognitive impairment, cardiovascular disease and premature mortality (Butler, Rehm & Fischer, 2017). Drug addiction is not only a public health burden to patients, but also to society. The cost of treatment for drug addiction would be much less expensive than the $600 billion dollars spent annually trying to reduce the associated healthcare and social costs of drug addiction (CDC, 2020). Therefore, having successful treatment options would be beneficial in reducing economic costs. Current treatment options include behavioral therapy and medication but have

approximately a 30% success rate. Relapse rates for drug addiction are 40-60% and are comparable to other chronic illnesses such as hypertension and asthma (NIDA, 2018). While relapse in an individual with drug addiction does not indicate that treatment is failing, pharmacotherapy targeting mechanisms that underlie relapse may be beneficial in increasing the success rate of treatment options when used in combination with current treatment options. Since relapse is triggered when an individual is exposed to drug-associated cues, successfully interfering with memory to weaken cocaine-associated memories in users would be a promising form of treatment.

Impairing reconsolidation of a cocaine-cue memory through a HAT inhibitor

The lateral nucleus of the amygdala (LA) is important for forming and storing cocaine-cue memories (Rich, Huang & Torregrossa, 2019). Interfering with signaling cascades in this region has shown to impair the ability to form and store cocaine-related memories (Wan, Torregrossa, Sanchez, Nairn & Taylor, 2014). Extinction and reconsolidation are two processes that can modify existing memories. Extinction is the process in which a conditioned stimulus (cocaine-cue) is present without the unconditioned stimulus (cocaine). In this way, a new memory is formed where the conditioned stimulus no long stimulates a conditioned response (pressing a lever to receive cocaine). It is important to note that spontaneous recovery can happen after extinction. Spontaneous recovery is the process by which conditioned response can be observed after extinction of a conditioned stimulus. Reconsolidation of a memory is when a memory enters a destabilized state after consolidation. This destabilized state allows memories to be updated with new information to strengthen or weaken the memory before being restabilized (Monsey, Ruiz & Taylor, 2020). Impairing the reconsolidation of cue-memories in animals has shown to be successful (Lonergan et al., 2015; Saladin et al., 2013; Sorg, 2012).

Garcinol is a HAT inhibitor of p300/CREB-binding protein (CBP) and p300/CBP associated factor, which have been shown to play important roles in reconsolidating auditory fear memory. Garcinol modulates epigenetic processes in the LA and impairs memory reconsolidation as a result (Maddox, Watts, & Schafe, 2013). Therefore, Monsey et al. wanted to explore the effect garcinol on memory specific to cocaine cues (2020). To explore garcinol's effect on cocaine cues, adult male rats were put in a chamber with an active and an inactive lever. The active lever administered cocaine along with a cue light and tone shortly after. The inactive lever did not administer cocaine or cues. Rats self-administered cocaine for one-hour sessions for 12 days. Following self-administration, rats underwent extinction for eight days where pressing either lever resulted in nothing. They underwent a reactivation session 24 hours later where they were put into a novel chamber that smelled like lemon, had different lighting and different floor textures so that the rat would not associate cocaine-cues with the new environment. Rats were presented with cocaine cues three times without any levers present. Rats were then given garcinol or vehicle intracranially into their LA (intra-LA) and were returned to their original chamber 24 hours later for a cue reinstatement test. Results showed that rats given garcinol intra-LA pressed the active lever less during reinstatement as compared to rats given vehicle. There were no significant differences in lever presses between the garcinol or vehicle group during acquisition or extinction (Monsey, Ruiz & Taylor, 2020).

To test whether reactivation of cocaine-cue memories is needed for garcinol to properly reconsolidate memories to impair cocaine-cue memories, researchers had a no reactivation group. The rats underwent self-administration, extinction, and reinstatement as described above. Compared to the reactivation group, the no reactivation group were still put in a novel chamber 24 hours after extinction, but the difference is that they were not presented any cues. One hour

after this session, rats received garcinol or vehicle. The cue reinstatement test showed that there were no differences between the rats who were given garcinol or vehicle. This suggests that reactivation in combination with garcinol is important for impairing cocaine-associated memories (Monsey, Ruiz & Taylor, 2020).

Arc and Egr-1 are immediate early genes (IEGs) that have been shown to be important for memory reconsolidation. Rats underwent reactivation and no reactivation and were systemically administered garcinol or vehicle. Thirty minutes after reactivation or no reactivation, rats were sacrificed, and researchers used RT-qPCR to look at Arc and Egr-1 in the LA. Results showed that rats who underwent reactivation and were given garcinol showed a significant decrease in the mRNA of both IEGs as compared to rats who received vehicle. Rats who underwent no reactivation showed no differences between garcinol or vehicle groups. These results suggest that systemic administration of garcinol after reactivation decreases IEG expression, leading to impaired cocaine-associated memory due to the decrease in lever presses seen in garcinol reactivation groups (Monsey, Ruiz & Taylor, 2020).

Since garcinol is a HAT inhibitor of CBP/p300 and PCAF and previous studies have shown that CBP/p300 is responsible for acetylating lysine residues 18 and 27 at histone 3 (H3K18 and H3K27) (Jin et al., 2011). Researchers wanted to see if this would be decreased following garcinol administration after reactivation. To do this, rats were sacrificed 90 minutes after either memory reactivation and no reactivation then a western blot was performed on the LA. Levels of acetylation of H3K27 were significantly reduced in rats receiving garcinol after reactivation as compared to vehicle, but acetylation of H3K18 did not show a significant difference between the groups. There were no significant differences between the garcinol or vehicle group in the no reactivation group. This suggests that garcinol specifically reduces

acetylation at H3K27 which has shown to be important for memory (Monsey, Ruiz & Taylor, 2020).

To see if they could bidirectionally regulate reinstatement, researchers used trichostatin A (TSA), a HDAC inhibitor. One group was given systemic administration of garcinol or vehicle intraperitoneally 30 minutes after reactivation and were given an additional injection of TSA or vehicle 15 minutes later. Results show that rats given vehicle and TSA had an increase in total active lever presses as compared to rats who were given just vehicle. Rats who were given garcinol and then vehicle had a decrease in total active lever presses as compared to rats who were given garcinol and then TSA (Monsey, Ruiz & Taylor, 2020). This suggests that TSA can allow for reinstatement of a memory even after given garcinol, showing that reinstatement can be regulated bidirectionally and that histone acetylation is an important regulator for cocaine-associated memories.

The use of glucocorticoid antagonists to impair memory

Exposure to drug-related cues can precipitate increases in glucocorticoids (Sinha, Fuse, Aubin & O'Malley, 2000). Glucocorticoids play an important role in memory and regulate the motivation to seek and take cocaine (Stringfield, Higginbotham & Fuchs, 2016). The basolateral amygdala (BLA) is comprised of glucocorticoid receptors and has been shown to have a role in contextual memory reconsolidation, promoting subsequent cocaine-seeking behavior (Fuchs, 2005). Thus, Stringfield et al. wanted to investigate the role of glucocorticoids in drug context-induced cocaine seeking behavior (2016). To do this, male rates underwent surgery to have catheters implanted into their right jugular vein as well as cannulas placed into the BLA or the posterior caudate putamen (pCPu). The pCPu group was used as a control to look at the specificity of BLA's role in drug context-induced cocaine seeking behavior. Rats were trained to

self-administer cocaine using a lever for two-hour sessions. Training occurred in two distinct environments: Context 1 had a red house light, intermittent pure tone, pine-scented air freshener and wire mesh flooring while context 2 had an intermittent white stimulus light above the inactive lever, a continuous pure tone, vanilla scented air freshener and a ceramic tile. Rats underwent extinction training in the context that they did not self-administer cocaine in. For example, if a rat self-administered cocaine in context 1, they underwent extinction in context 2. For reinstatement, either 3 ng of mifepristone, 10 ng of mifepristone, or vehicle was infused into the BLA. These doses were based on their ability to inhibit memory consolidation (Roozendaal and McGaugh, 1997) and reconsolidation as described in previous studies (Jin et al., 2007). Rats were then placed into the cocaine-paired context for one hour. Here, both the active and inactive levers were present but pressing them resulted in no infusions. Rats who were given intra-BLA infusions of 10 ng mifepristone showed less active lever presses in the cocaine-paired context than both the 3ng mifepristone and vehicle group, with the vehicle group showing the most active lever presses. As hypothesized, administration of 10 ng mifepristone in the pCPu showed no significant differences, showing that 10 ng mifepristone works to attenuate cocaine seeking behavior in the cocaine-paired context specifically in the BLA (Stringfield, Higginbotham & Fuchs, 2016).

To test mifepristone's effects on cocaine-memory reconsolidation an additional study was done by Stringfield et al. (2017). Male rats underwent the same procedures as described above. However, to test cocaine-memory reconsolidation, rats were either put in the previously cocaine-paired context to become the reactivation group or were put back into their home cages to become the no reactivation group. During this session, active and inactive levers were present but had no outcomes if pressed. Immediately after the reactivation or no reactivation session, rats

received either vehicle, 3 ng or 10 ng mifepristone intra-BLA. Observations of the reactivation group showed that the 3-ng mifepristone group had more active lever presses than the vehicle group when put back into the cocaine context while the 10-ng mifepristone group did not. The no reactivation group showed no significant differences between the groups. To show specificity of BLA's role in cocaine-associated memories, 3 ng or vehicle was given to the posterior caudate putamen (pCPu) of mice. Mice given intra-pCPu after memory activation showed active lever pressing comparable to mice given vehicle. Mice given 3 ng of mifepristone who did not undergo reactivation showed more active lever presses than the vehicle group (Stringfield, Higginbotham, Wang, Berger, McLaughlin & Fuchs, 2017). Taken together, these results suggest that reactivation is important in reconsolidating memories in order to interfere with them. Furthermore, infusion of high-dose mifepristone is effective at impairing cocaine-associated memories and it's affects occur specifically at the BLA.

Corticosterone is the main glucocorticoid in rodents (Katsu & Iguchi, 2016) and because of this, Stringfield et al. wanted to measure corticosterone levels which was done by taking various blood samples at different time points (2017). Corticosterone levels were taken before extinction session seven and immediately after. They were also taken 24 hours before the memory reactivation session and immediately after exposure to the cocaine-paired context or after exposure to the home cage. Samples were also taken 30, 60, and 90 minutes after either 3 ng or 10 ng intra-BLA mifepristone, or vehicle. It was observed that post-session serum corticosterone levels were higher 30 minutes following rats who underwent the reactivation session as compared to rats who did not. However, serum corticosterone concentrations were increased only in rats who were given 10 ng mifepristone relative to baseline, but not rats given 3 ng of mifepristone or vehicle. In addition, there were no significant increases in corticosterone

levels across groups in rats put into their home cage (Stringfield, Higginbotham, Wang, Berger, McLaughlin & Fuchs, 2017). These results show that re-exposure to the cocaine context increases corticosterone levels which act on glucocorticoids to result in an increase of active lever pressing in these rats indicating that re-exposure to the cocaine context may be a stressor that causes an increase in cocaine seeking. Administration of 10 ng of mifepristone in rats re-exposed to the cocaine-context resulted in an increase of corticosterone levels, but a decrease in active lever presses was observed. Therefore, the high dose of mifepristone may act to downregulate glucocorticoid receptors d the increase in corticosterone levels.

DISCUSSION

The findings discussed in this review suggest that chronic cocaine-use leads to alterations in proteins involved in regulating memory processes. These alterations in proteins occur via epigenetic modifications of the genes that are responsible for expressing these proteins. Memory plays an important role in the risk of relapse in that relapse is triggered by cues that remind an individual of taking a drug. Gaining knowledge of how proteins involved in memory processes are altered following cocaine-use is beneficial in understanding the underlying mechanisms involved as well as beneficial in helping to find a potential treatment for cocaine-use disorder.

The studies presented here did an excellent job at eliminating confounding variables such as making sure that a decrease in active lever presses was not due to impairment in learning acquisition. However, controlling for the environment too much does raise an issue in that it is impossible to control for all confounding variables in human studies. Therefore, these results may not be representative in regard to humans.

The research described above uses male rats as their subjects. While rodents are great models for exploring addiction, the underlying mechanisms and pathways may not be fully translatable to humans. Since there are some complexities to studying female rodents such as accounting for the estrous cycle, research studying female models of addiction is not as ideal. However, studying understanding how drug addiction affects females is beneficial. Women seem to have a greater reinforcing efficacy in response to cocaine as compared to males which in faster progression of the addiction as well as higher rates of relapse. Behavioral differences between the sexes are thought to be a major contributor to this divergence in humans. Studies show that,

in general, women use drugs in response to anxiety or depression, while males use drugs as a result of impulsivity. However, there is thought to be a biological effect at play as well. The exact biological cause is unknown, but it is thought that estradiol plays a role in this sex difference as estradiol has been found to act on dopamine receptors ("The Neuroscience of Cocaine", 2017). Thus, understanding the mechanisms underlying this sex difference would be advantageous in implementing personalized treatments for drug addiction.

RECCOMMENDATION FOR FUTURE STUDIES

Future studies should aim to look at female models of drug addiction to gain a better understanding of how drug addiction may exert its effects in a sex-dependent way. While drug addiction in men is more likely to result in an overdose, women may be more susceptible to craving and relapse (National Institute on Drug Abuse, 2020). Exploring sex differences in drug addiction would be paramount in providing personalized treatment options. It is well known that the HPA-axis has a negative feedback loop which may be the cause for why the high dose of mifepristone results in an increase in corticosterone levels, but a decrease in active lever presses. However, the mechanisms of mifepristone's exact affects need to be studied further.

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