Induced Methylation of the KITLG as a Treatment for Hippocampal Degeneration in Bipolar Disorder

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Abstract

This article is an examination of the literature regarding bipolar disorder and our understanding of it. Specifically, a focus is placed on the biological symptoms associated with bipolar disorder and their impact on the hippocampus. Through this research, a new genetic component will be introduced as a link between bipolar disorder and hippocampal degeneration. It appears that methylation of a specific ligand that mediates the stress response can be used as a treatment for the neurotoxic effects of cortisol. By reducing the high levels of cortisol found in those with bipolar disorder, brain damage to the hippocampus may be reduced. This treatment could be performed through induced methylation of the proposed ligand.

Induced Methylation of the KITLG as a Treatment for Hippocampal Degeneration in Bipolar Disorder

Bipolar disorder is a mood disorder classified by two distinct phases: manic and depressive. A specific ligand, the KITLG, has been shown to play a role in mediating the stress response (Houtepan et al., 2016). Under ideal conditions, this ligand becomes methylated in times of childhood adversity to mediate the stress response (He et al., 2019). When this does not happen, it serves as a risk factor for bipolar disorder (Wrigglesworth et al., 2019). Mania, a symptom of BD, has been shown to increase corticosterone levels in rats (Abulseoud et al., 2017). High levels of cortisol, the human stress hormone equivalent, have been shown to reduce hippocampal volume (Li et al., 2022). Cushing's disease provided the model for this discovery. Similar effects were also found in those with BD-I, BD-II, and major depressive disorder. Hippocampal damage was more severe in those with BD-I, a type of BD known for its more severe manic episodes (Cao et al., 2017). Therefore, it could be inferred that the increased

cortisol produced by greater manic episodes results in reduced hippocampal volume. It is possible that the KITLG could be of use in slowing the effects of hippocampal degeneration.

He et al. (2019) outline the connections between the KITLG and bipolar disorder in regards to childhood adversity. The researchers collected 91 control patients and 50 bipolar patients and assessed them for childhood adversity and bipolar disorder. The 141 subjects then had their blood taken which was then analyzed through a series of epigenetic processes to determine the level of methylation of the KITLG. The results showed a significant decrease in methylation in those with bipolar disorder. Furthermore, there was no correlation between childhood adversity and KITLG methylation in bipolar patients, yet a positive correlation existed within the control group (He et al., 2019). All in all, these results indicate that lack of methylation of the KITLG is a risk factor for bipolar disorder. The research provides a sturdy baseline and establishes a connection between methylation of the KITLG and bipolar disorder. In addition, it also leads into the second article which discusses biological implications of an unmethylated KITLG.

Wrigglesworth et al. (2018) focused on finding the interactions between methylation of the KITLG and cortisol in the body. Similar to the first study, 142 participants had their blood collected and DNA methylation analysis was performed to determine methylation levels of the KITLG. Cortisol levels under basal and stressful conditions were determined through radioimmunoassay analysis in the morning, day, and evening. Results found a significant negative correlation between KITLG methylation and morning cortisol levels during stress (Wrigglesworth et al., 2018). As the KITLG became methylated, cortisol levels under stress decreased. The researchers conclude that the KITLG being methylated may reduce the body's stress response by decreasing the amount of cortisol secreted. This research establishes a

biological mechanism, the stress response, that can be altered as a direct response to an unmethylated KITLG. Following the research by He et al. (2019), a connection is now established between how childhood adversity can lead to methylation of the KITLG which is responsible for stress regulation. Furthermore, when this ligand is not methylated, it serves as a risk factor for bipolar disorder.

An article by Abulseoud et al. (2017) deals with inducing a manic episode in a rat and determining their corticosterone levels. Here, researchers used hypothalamic lateral kindling to create a manic-like episode for the rat. At the same time, blood samples were collected from the kindled group, a sham group with electrodes implanted but not activated, and a control group. Results showed a very large spike in corticosterone in the kindled group during the stimulation, and no significant change for the sham group nor the control (Abulseoud et al., 2017). Here, a connection is established between corticosterone, the stress hormone in rats, and mania, one-half of bipolar disorder. Building on the previous two articles, people with bipolar disorder are less likely to have a methylated KITLG following childhood adversity. If the KITLG doesn't methylate, the individual can feel the amplified effects of bipolar disorder. Since mania increases stress hormone levels further, the effects are exacerbated.

Li et al. (2022) investigates the effects of high cortisol levels on the hippocampus's volume. The researchers used an MRI to compare hippocampus volume between those with Cushing's disease to a control group. Cushing's disease is marked by an individual's chronic hypercortisolism, meaning that they have consistently high levels of cortisol. It was found that all four hippocampal subregions that were investigated were smaller in the CD subjects compared to the control group. Taking the previous articles into account, cortisol's neurotoxic effects become clear. Prolonged exposure to high levels of cortisol degenerate the hippocampus, and manic

episodes increase the amount of cortisol produced as well. Both the KITLG and manic episodes increase cortisol levels that appear to cause hippocampal damage as shown by CD subjects.

Cao et al. (2017) serves to reinforce the claims of the previously discussed article, as well as connect it back to the research by Abulseoud et al. (2017). Cao et al. also sought out differences in hippocampal volumes, just as Li et al. (2022) did. However, Cao et al. focused less on the effects of cortisol and placed a greater emphasis on the psychological disorders associated with a smaller hippocampus. The researchers recruited subjects with BD-I, BD-II, major depressive disorder (MDD), as well as a healthy control (HC) group. They then utilized an MRI scanner to determine the size of different regions of the hippocampus and began to compare them. Statistical analyses showed that the MDD and HC groups were not significantly different when it came to hippocampus volume. However, those with BD had a significantly smaller hippocampus than the other groups. (Cao et al., 2017) Tying it all together, the pathway becomes clear. The KITLG, faultily unmethylated following childhood trauma, increases the body's overall cortisol response. This pairs with the manic symptoms found in bipolar disorder that also produce excessive stress hormone. All of this cortisol buildup results in neurotoxic effects as seen in those with CD. Finally, the patient with bipolar disorder suffers from a reduced hippocampus. Therefore, it can be inferred that childhood trauma can exacerbate effects of bipolar disorder, specifically accelerating the rate at which the hippocampus shrinks through a lack of methylation of the KITLG. Were the KITLG to be methylated, the effects could potentially be mitigated, resulting in slowed degeneration of the hippocampus. As such, it becomes necessary to analyze this possibility further and with greater depth than previously discussed.

Analysis

Bipolar disorder, or BD, is a severely debilitating disorder that impacts many lives. He et al. (2019) conducted their research into childhood adversity to discover the pathogenesis of bipolar disorder with the hope of furthering research towards a cure or other treatments. They began their operations with the knowledge that childhood adversity is a risk factor for the development of mental disorders, such as bipolar disorder (Aas et al., 2016, as cited in He et al., 2019). The researchers also knew that DNA methylation can cause biological changes that can result in mental disorders (Ludwig & Dwivedi, 2016). He et al.'s research combined these two points.

He et al. examined the DNA of individuals diagnosed with bipolar disorder. Specifically, they examined the methylation of the KITLG when faced with childhood adversity. To begin their research, a sample of 91 control subjects, along with a sample of 50 patients that were diagnosed with BD was collected. Two variables had to be examined, Childhood Trauma and DNA Methylation. To calculate the Childhood Trauma, participants were assessed using the Childhood Trauma Questionnaire. The CTQ had 24 valid questions that assessed feelings of childhood abuse, both physical and sexual, and feelings of neglect. These scores were tallied and used to compare the two groups. The process of DNA Methylation, however, is much more complex than a simple survey. A simple commercially available kit was used to draw the participants blood. From there, a Zymo Kit was used to perform the bisulfite conversion required to separate the DNA. Bisulfite conversion is a process where DNA is split and the cytosines are turned into uracils. Cytosines that are methylated are unaffected by the process. Next, riboGreen, a fluorescent dye, was used to quantify the amount of RNA that was collected following the bisulfite conversion. The dye works by binding to nucleotides and if there weren’t enough nucleotides, the fluorescent glow would be diminished. With a strong enough glow, the

researchers knew they had collected a sufficient amount of RNA. Then, BioAnalyser was used to determine the integrity of the collected sample. BioAnalyser is an electrophoresis technique that separates the RNA based on size and charge using electrodes. At this point, the KITLG has been isolated. Following the electrophoresis, BeadChip data is collected. BeadChip, or BeadArray technology uses many silica microbeads containing numerous genotypes to isolate specific parts of a gene. These microbeads connect to specific nucleotides along the KITLG, isolating its components even further. Penultimately, specialized software called GenomeStudio Software was used to analyze the results of the BeadChip data. The software could determine just how much of the KITLG was methylated and in which areas. Finally, results were estimated using the Houseman procedure. The procedure takes data from peripheral blood, found in the veins and arteries, and corrects the results to be better indicative of whole blood found within the body. Though incredibly complex, the means of assessing DNA methylation is thorough and necessary to understand how data was collected from participants.The study conducted yielded powerful and beneficial results. The BeadChip data showed that there was a significant difference between the KITLG Methylation of those with BD and the control group. Those who had BD had, on average, lower levels of KITLG Methylation. The difference was also found to be independent of the various medications the BD group was on. Surprisingly, there was no significant association between KITLG Methylation and Childhood Adversity in the BD group. The control group, however, had a positive association between KITLG Methylation and Childhood adversity. When reconsidering the data from the BD patients, it could be inferred that BD could develop from a lack of KITLG Methylation.

The research done by He and his team concluded that methylation of the KITLG in the face of adversity protects an individual from developing bipolar disorder. Another question is to

discover what methylating the KITLG does biologically for an individual. How does the KITLG regulate the body in such a way that leads to BD? Luckily, the following research by Wrigglesworth et al. (2018) addresses this question, and results suggest that they lie within the body's stress response system.

To further understand the KITLG and its effects, Wrigglesworth et al. devised a plan to research the biological differences between those with and without a methylated KITLG. Researchers believed that the KITLG was responsible for regulating the body's stress response to cortisol. Cortisol is the body's stress hormone, and is involved in feelings of anxiety and regulating the fight or flight response. Too much of it can cause weight gain, brain damage, osteoporosis and mood swings (Society for Endocrinology, 2019). If the KITLG were capable of influencing this system, understanding its effects would be crucial for the treatment of those with cortisol-related disorders, such as anxiety or bipolar disorder.

To verify their hypothesis, the team needed to collect data. Community members from Montpellier France were sampled and 142 were recruited in total. The sample consisted only of members over the age of 65. Once consent forms had been signed, the steps of determining KITLG methylation could begin. The procedure was similar to the process used by He et al., but varied in some areas. First, Wrigglesworth et al. collected whole blood samples, not peripheral blood, which made the Houseman procedure unnecessary. Once again, bisulfite conversion was used to separate the DNA. While the machine and technique were different, the end result was the same. Next, the team targeted the KITLG region and used a Polymerase Chain Reaction (PCR) to amplify the KITLG region. PCR is a mechanism by which specific parts of a gene are selected for and replicated by enzymes to produce more of the desired chain allowing more samples to be acquired from less base material. Finally, methylation of the KITLG was assessed

through use of the SEQUENOM MassARRAY EpiTYPER platform, which could determine methylation status by difference in weight. From this, the team was able to collect sufficient data regarding the methylation of the KITLG. The second part of the study involved cortisol. To determine cortisol levels, participants were recorded both at home, a basal condition, and in a clinical exam setting, a stressful condition. The subject's saliva was then analyzed for cortisol concentration under these conditions at different times throughout the day and diurnal cortisol levels were determined by using the area under the curve (AUC). Methylation of the KITLG and diurnal cortisol levels were then compared. From this comparison, a significant negative association was discovered. Data showed that as levels of KITLG methylation increased, diurnal cortisol levels decreased under the stress condition. A roughly 25% reduction was found as a result of the KITLG methylation. The association was also found in morning cortisol levels analyzed independently, but the same did not hold true for evening cortisol levels. Worth remembering, however, is that evening cortisol levels are highly affected by the events of an individual's day, and therefore would likely vary.

The results of the aforementioned studies can lead us in the right direction to answer this last question. With the data provided by Wrigglesworth, it has been established that methylation of the KITLG can reduce the amount of diurnal cortisol in the body. Adding this to He's discovery regarding childhood trauma and the KITLG, a new interpretation can be made. Typically, the KITLG methylates following negative experiences in childhood. This then regulates the body's response to stress. Individuals without a methylated KITLG produce greater amounts of cortisol and are subjected to greater stress as a result. All that is left is to establish a link between feelings of stress and the emergence of bipolar disorder.

One of the brain areas responsible for the symptoms of bipolar disorder is the hypothalamic-pituitary-adrenal axis (HPA axis). The HPA axis, among other purposes, serves as the body's central stress response system. In addition, those who are diagnosed with bipolar disorder frequently have overactivation within this area (Belvederi Murri et al., 2016). To further establish this link, Abulseoud et al. (2017) set out to simulate manic responses within rat models. The data would allow them to analyze the changes to the HPA axis during a manic episode and use those findings to further research in humans. Specifically, Abulseoud's team wanted to analyze the corticosterone levels of rats undergoing a manic episode and see how it impacts the rest of the HPA axis.

In order to induce a manic episode, an operation called bilateral lateral hypothalamic kindling (LHK) was used. LHK involves stimulating the lateral hypothalamus of the rat using electrical current. To prepare the rats, a stereotaxic apparatus was used to find the lateral hypothalamus. Here, the bipolar stimulating electrodes were inserted and adhered with dental cement and three screws. If the implantation went awry or the rat showed signs of brain damage, it was humanely euthanized to avoid cruelty and ensure validity of the results. The process was repeated for two groups of rats. These groups were the Sham group, which would receive no stimulation, and the Kindled group, which would have the electrodes activated. A third group without any electrodes implanted was added as well and served as the Control group. In order to determine the amount of corticosterone present within each group, a cannula was surgically inserted into the right external jugular vein. The cannula would allow blood to be collected, which could then be assessed for corticosterone concentration, and saline to be pumped in as a replacement. To induce mania, a 10 second pulse of 180 Hz and 200 μs pulse width was applied with 30 seconds of rest following afterwards. The pulse, performed 10 times consecutively,

constituted a train. Seven trains were applied with 2 minutes of rest being used between individual trains. These pulses induced manic behaviors, as expected by the researchers. Then, 31 blood samples were collected over the next 24 hours at a rate of roughly once every hour. The final samples, however, were collected within 10 minutes of each other. After the blood had been collected, the animals were euthanized and proper implantation of the electrodes was verified in the brain. The results showed approximately a 3 times increase in corticosterone concentration following the implementation of LHK in the Kindled group compared to both the Control and Sham groups. LHK induced-mania caused the rats to release greater amounts of their stress hormone. A small overall increase was also observed in the Sham group, causing greater amounts of corticosterone compared to the Control group. However, the Sham group was still eclipsed by the Kindled group immediately following the stimulation. Interestingly, the amount of corticosterone in the Kindled group was lowest of all groups a few hours after the mania-induction. Therefore, stimulating the lateral hypothalamus can induce mania, which increases the concentration of corticosterone present within a subject temporarily.

Using this revelation, it can be concluded that corticosterone can be viewed as a marker for increased HPA axis activity. Additionally, the presence of increased corticosterone within the Sham group implies that the implantation of an electrode on its own can influence the excitability of neurons in that area. As the process of mania-induction verified, overstimulation of the HPA axis can induce manic episodes which results in a greater concentration of stress hormone. Applying these results to humans, it could be inferred that a symptom of bipolar disorder, specifically mania, could be an increase in cortisol concentration in individuals. The detected change in cortisol concentration aligns with the findings of the previous studies and

possibly is the result of the KITLG. However, it is also important to consider the effects of long term exposure to increased levels of cortisol in a person.

In order to investigate the effects of consistent cortisol, a model is needed. Fortunately, one can be found in the form of a rare disease. Cushing's Disease (CD) is a disorder "characterized by chronic hypercortisolism" (Li et al., 2022) and will serve as a good model of what happens to the brain in the presence of excessive cortisol. Li et al. knew that stress could cause damage to the hippocampus and therefore used CD patients to research the effects of cortisol specifically. Using MRIs, they compared hippocampal size in four areas between healthy controls and subjects with CD. With this knowledge, the team sought to suggest more effective treatments for those with CD. As established previously, high cortisol levels are likely found in those with BD as well. Therefore this research's findings could transfer between the two groups. To begin, 100 participants were selected for the study. 47 were verifiably diagnosed with CD by experienced endocrinologists and the other 53 served as healthy controls (HCs). Similar to Wrigglesworth et al., the participants had their cortisol levels measured throughout the day. Levels of 24 hour, urinary-free cortisol (24hUFC) levels were recorded thrice, once every 8 hours. Following collection, cortisol content was measured using an ADVIA Centaur Analyzer. This was done for the 47 CD participants as well as the 53 HCs. Both groups were also subjected to structural and functional MRIs in order to gather images regarding the size of their hippocampal areas. This would allow each group's hippocampal size to be compared. The data was acquired using a 3.0-Tesla MR system and then analyzed using a sagittal Fast Spoiled Gradient-Echo (FSPGR) sequence in order to produce the 3D structures. The system interpreted the data and made it easier to perceive. Preprocessing of the resting-state fMRI images was handled through the use of SPM12 and Data Processing Assistant for Resting-State fMRI. This

SPM software would allow the fMRI images to be statistically compared visually. To simplify, the fMRIs took pictures of the participants' brains, the FSPGR compiled the images and made them clearer, and the SPM software compared the results. In addition, a two-sample t-test was performed to determine the differences in 24hUFC levels. The results revealed that levels of 24hUFC were significantly higher ($p<0.001$) in the CD group than the HC group. Across the whole day, the CD group had approximately 10 times more cortisol in their system than the HC group. Analysis of the hippocampus was split into 4 areas: left rostral, left caudal, right rostral, and right caudal. For all areas except the left rostral hippocampus, the CD group's size was significantly smaller ($p<0.001$) than the HC group. For the left rostral hippocampus, results showed that the CD group was still significantly smaller than the HC group, but only to a p of 0.001 instead of less than 0.001. Overall, Li et al. revealed that incredible amounts of cortisol for extended periods of time can lead to hippocampal degradation.

Based on the previous four articles, a pattern can be established. He et al. (2019) and Wrigglesworth et al. (2018) introduce the KITLG and explain how it originates and what it can do. In the face of childhood adversity, the KITLG can methylate. When this happens, a person becomes less likely to develop bipolar disorder since the gene is responsible for regulating cortisol production. As demonstrated in rats by Abulseoud (2017), high levels of corticosterone can result from induced mania. When people have high levels of the stress hormone, cortisol specifically, it begins to reduce the size of their hippocampus. It would appear that failure to methylate the KITLG following childhood adversity serves as a risk factor for bipolar disorder. The manic episodes involved in BD then result in high levels of cortisol which degrade the hippocampus. While these findings are interesting, it still has to be established whether or not they are truly present in those diagnosed with bipolar disorder.

Fortunately, research has been done investigating correlations between hippocampal size and bipolar disorder). For example, Cao et al. (2017) worked to investigate the relationship between hippocampal size and various mood disorders. There had been conflicting information in the field at the time, and they wanted to sort it out. They used healthy subjects, subjects with major depressive disorder (MDD), and subjects with BD. A similar process was used to gather data to that of Li et al., but the hippocampus was divided differently. It was hypothesized that both MDD patients and BD patients would have smaller hippocampi than the healthy subjects. However, the researchers believed that the manic component of BD would result in greater hippocampal degradation compared to the MDD group. If this were to hold true, it would establish a link between high levels of cortisol, as a result of mania, and reduced hippocampal volume. The participants in this study had to be diagnosed with either BD-I or BD-II or with MDD. Healthy controls were collected as well and their mental health was evaluated prior to testing. Three hundred seventy-one spell out numbers that begin a sentence subjects were recruited overall. One hundred fifty-two (spell out) were HCs, 133 were diagnosed with BD I or II, and 86 were diagnosed with MDD. A 1.5 Tesla MRI scanner was used to establish a structural scan of each participant's hippocampus. FreeSurfer software version 5.3.0 was used to correct the images collected and construct the hippocampus from the images. A separate algorithm was used to split the left and right sections of the hippocampus into eight divisions per side. Then, an additional protocol was used to remove outliers or unclear data to ensure results were high quality. Once again to summarize, the MRI took pictures, the FreeSurfer software made them clearer and created a 3D model, and finally an algorithm split the model into 16 total parts, eight on the left, and eight on the right. This whole process was then cleaned up through an additional protocol. However, no pictures were excluded as the protocol deemed them all of considerable

quality. To analyze the results, a general linear model was used as well as post hoc t-tests. The results were as the researchers predicted. Hippocampal volumes overall were significantly ($p=0.002$ left, $p=0.011$ right) reduced in those with mood disorders. However, once split by subsection, not all areas were significantly different. Between the BD and HC groups, 4 of 8 left subsections were significantly smaller in the BD group and only one of the right subsections were significantly smaller. When comparing the BD and MDD groups, a similar effect is found. The same 4 of 8 left subsections are significantly smaller in the BD group, and 2 of the 8 right subsections are significantly smaller for the BD group. It is worth noting, however, that the BD group had reduced volume in all subsections in all cases. Most interestingly was an observation regarding the driving force behind the BD group. Cao et al. claim that "the lower hippocampal subfield volumes of BD were majorly driven by patients with BD-I" (Cao et al., 2017). Results showed that those with BD have a reduced overall hippocampal volume, just as predicted.

Discussion

Upon reviewing the aforementioned studies and their results, it becomes necessary to view them all together. Bipolar disorder occurs in two forms, BD-I and BD-II. The major diagnostic difference occurs with the severity of a person's manic episodes (Mayo Clinic, 2022). Those diagnosed with BD-II are referred to as hypomanic. This means that their manic symptoms are reduced or shorter in duration. For those with BD-I, they experience full manic episodes, which are more severe. If significantly reduced hippocampal size for those with BD was largely carried by those with BD-I as Cao et al. claim, it would reinforce the ideas of Li et al. (2022) and Abulseoud et al. (2017). Manic episodes produce greater amounts of corticosterone in rats (Abulseoud et al., 2017). High amounts of cortisol have been shown to reduce hippocampal volume in those with Cushing's Disease (Li et al., 2022). Similar results have been found in

those with BD and these findings were largely the result of those with BD-I (Cao et al., 2017). Therefore, the manic part of bipolar disorder is likely the driving force responsible for a direct decrease in overall hippocampal volume.

With manic episodes and cortisol reducing the size of the hippocampus, it's best not to forget the KITLG and its role. While under a stressful condition, the body produces cortisol. This production is controlled in part by the KITLG. When this gene is methylated, the amount of cortisol produced is decreased under stress. The body can naturally methylate this gene following a traumatic childhood, which reduces the body's response to stressful events. When the body does not methylate the KITLG following a stressful event a person produces more cortisol when under stress. It is possible that this increased cortisol production also occurs during a manic event. Prolonged exposure to cortisol as produced during a manic episode leads to reduced hippocampal volume. Were the KITLG to be methylated in a population with bipolar disorder, it is possible that the effects of their mania would not be as severe.

With all of this information in mind, I believe that induced methylation of the KITLG could be used to slow the progression of hippocampal degeneration as a result of the neurotoxic effects of cortisol. Were the KITLG to be methylated, it is possible that conditions could improve for those with bipolar disorder. Cortisol production would slow which would reduce the presence of excess neurotoxic chemicals in the hippocampus.

Future Research

In order to verify that such a treatment would work, additional research would have to be done. The first thing to do would be to investigate the specific pathway used to produce excess cortisol during a manic episode and what role the KITLG plays in that pathway. Not all people

with BD have an unmethylated KITLG. In some cases it seems that BD can overpower the regulatory effects of the KITLG. However, as established previously, methylation in those with BD is still significantly lower than in those without. Perhaps the mediating effects of the KITLG are only effective when it is methylated early. An experiment could be done comparing the ages of subjects with a methylated KITLG and their differing 24hUFC levels to see if age plays a role. Understanding how the KITLG works and when it methylates is crucial to unlocking its potential restorative effects.

Additional research could also be done into what causes the body to methylate the KITLG in the first place. Not all people who endured childhood adversity underwent methylation of the KITLG. Differences in environment or perhaps other factors altogether could influence the methylation of the KITLG. Knowing the specific circumstances that lead to methylation could help with treatment. Knowing what causes it can lead to inducing it. Being able to tell if someone's experience has likely met the 'criteria' for methylation could lead to more effective and quicker treatment strategies. Perhaps there exists a second gene working in tandem with KITLG that explains the difference in methylation between groups.

It is also important to note that the KITLG could potentially assist those with Cushing's Disease as well. If CD follows a similar pathway when releasing cortisol, then methylation of the KITLG could potentially reduce cortisol levels in those cases as well. Future research could investigate the KITLG methylation levels of those with CD in hopes that it could shine a light on KITLGs role in cortisol regulation overall. If they too have low levels of KITLG methylation then it could be inferred that induced methylation could help them as well. However, the causes of both CD and BD differ wildly, so even more research would need to be done.

Turning away from the KITLG, researchers could investigate the effects of hippocampal damage on the HPA axis. Perhaps the neurotoxic effects of cortisol are more widespread than initially understood. If the effects are more widespread, then it is possible that it is cyclical and creates a positive feedback loop. Increased cortisol levels could hypothetically damage the areas responsible for cortisol regulation. Since cortisol damages the brain, perhaps it impacts additional areas responsible for manic episodes and makes them worse over time. Rampant cortisol could potentially increase the frequency or severity of these episodes, releasing even more cortisol into the system. If this were true, then temporality would have to be researched as well. It would be pivotal to know which symptoms arise first and lead to which other symptoms. Understanding the relations between different brain areas and the effects they can have on each other provides valuable information on how BD can progress.

Still moving away from the KITLG, research could be done into reducing the neurotoxic effects of cortisol. If KITLG influences production of cortisol, then it stands to reason that some gene must regulate reception of cortisol. It is likely that yet another gene, or set of genes, should be responsible for removing or inactivating excess cortisol. Investigation into these systems and their respective genes provides another way to reduce the effects cortisol has on hippocampal degradation. Understanding how to inactivate cortisol faster could be just as effective as reducing its production overall.

Overall, there are many potential avenues for further research. Understanding the KITLG in greater detail would go a long way in accelerating treatment. Knowing what causes it to methylate could help us understand how to induce it ourselves. It could unlock more information about similar methylating effects in the body and what causes them. This knowledge could also help those with CD as well. A large number of disorders are caused by excessive cortisol levels

and understanding how to treat one could make it easier to treat others. Research can go beyond the KITLG as well. Further understanding of the effects hippocampal damage has on the brain and its associated systems is crucial for treatment. Rarely does a disorder affect just one part of the body. It is important to discover the effects that one symptom has on the body's other homeostatic relations. Cortisol has been the culprit of many of these problems and understanding other ways to limit its effects would be beneficial as well. Reducing its active time could reduce its neurotoxic effects and discovering other ways to regulate it beyond the KITLG would be great further research. Numerous studies can be done in the near future to understand and treat stress-related disorders more effectively.

Conclusion

In conclusion, this paper provides support for the idea that hippocampal degeneration associated with bipolar disorder could be reduced through induced methylation of the KITLG. This ligand can reduce the neurotoxic effects of cortisol by reducing its production throughout the brain. With lower cortisol levels, damage to the hippocampus should decrease. This would in turn reduce the harmful effects of bipolar disorder. Were this treatment to be researched further and implemented effectively, the results for those with bipolar disorder could be life changing.

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