PART 2: TWO SEPARATE AND DISTINCT POPULATIONS

This article accompanies the first article entitled: *Part 1: Behaving Differently* and the third article entitled *Part 3: A New Disease State - Definition and Treatment*

ABSTRACT

In the first accompanying article, we showed that the two groups, the opioid naive and the opioid dependent behaved differently in the setting of opioid abstinence. We raised the question whether the the proper diagnosis for the opioid dependent should be the mental health diagnosis of Opioid Addiction/ Opioid Use Disorder or rather if the proper diagnosis should be the neuroendocrine emergency known as Autonomic Dysfunction and due to genetic damage. The article concludes that the proper diagnosis is the neuroendocrine emergency known as Autonomic Dysfunction and due to genetic damage. That stated, the first article achieved the goal of demonstrating that the two groups exhibited different behavior in identical situations- opioid abstinence . From an epidemiological perspective, the next step is to establish that the two populations not only behave differently, but that they are in fact genetically two separate and distinct populations. If the two groups exhibit different behavior in identical situations and are recognizably distinct genetically, then we have established and defined a new disease state. By establishing a new disease state, the issue of a mental health disorder is permanently debunked. Here we show that Artificial Intelligence can establish with certainty that the two populations are separate and distinct. To the best of our knowledge, this is the first time that Artificial Intelligence has been used to establish a separate and unique population as being its own disease state. We also show that Artificial Intelligence can lead the way in the development of a DNA based clinical test. The era of denying these people the truthful diagnosis must come to an end. Recognition of the disease state and application of the correct diagnosis along with deployment of a DNA based clinical test to support the proper diagnosis are the goal of this second article.

1). INTRODUCTION

1.1). DISTINCT SCENARIOS REQUIRING RECOGNITION OF THE PROPER DIAGNOSIS OF AUTONOMIC DYSFUNCTION SECONDARY TO GENETIC DAMAGE DUE TO REPETITIVE OPIOID TOXICITY

1.1.1). ESTABLISHMENT OF THE INJURED AS VICTIMS OF A POISONING

This isn't an "illness". This is a poisoning. This poison was manufactured and distributed by an industry. And establishing these individuals as victims of a poisoning has implications even beyond a proper diagnosis and treatment. For just as health is a human right, so is justice also a human right. For these reasons, we look to Artificial Intelligence to conclude the diagnostic debate by establishing the two groups as genetically separate and distinct and provide a proper clinical test to substantiate the status of these individuals as victims of a mass poisoning.

1.1.2). REFUTATION OF AN IMPROPER DIAGNOSIS

Opioid Addiction/Opioid Use Disorder was not a diagnosis substantiated by science. However, an entire medical/pharmaceutical industrial complex has arisen on this unsubstantiated diagnosis. The unsubstantiated diagnosis has even infiltrated into the state and federal governments. It is against this medical/pharmaceutical industrial complex that the individual patient must now fight to simultaneously rid themselves of the inappropriate mental health diagnosis and establish the proper genetic damage diagnosis. Demonstrating that the two populations, the opioid naive and the opioid dependent, are separate and

distinct genetic populations and development of an accurate DNA test with both sensitivity and specificity is the proper pathway to ending the inappropriate mental health diagnosis and establishing the proper genetic damage diagnosis. To continue the mental health diagnosis without proper testing will be an act of medical malpractice. To continue to profit off of the manufacturing of medication based upon the debunked indication of Opioid Use Disorder is a perversion.

1.1.3). DETERMINING AT RISK STATUS FOR CATASTROPHIC CARDIOVASCULAR SEQUELA

In the accompanying article, we established an epidemic of Takotsubo Cardiomyopathy occurring in the same timespan as the Opioid Crisis. We further established an epidemic of cardiovascular deaths also occurring in the same timespan as the Opioid Crisis. This epidemic of cardiovascular deaths was of such a magnitude that US life expectancy was stalled. These cardiovascular epidemics are believed to share the common characteristic of being catecholamine toxicity driven. This catecholamine toxicity is a component of the autonomic dysfunction that occurs in individuals whom have suffered genetic damage due to the opioids and when opioid abstinence is attempted. The exact number of individuals at risk for a catastrophic cardiovascular event is unknown. Recognizing the genetically damaged population as unique and with its own risk factors associated is fundamental to ending these separate but intertwined epidemics. A screening test for those at risk for a catastrophic cardiovascular event would be a necessity to ending this epidemic of cardiovascular sequela.

1.1.4). STRENGTH IN LEGAL PROCEEDINGS

The opioid manufacturers and distributors at every level of the supply chain will be facing fallout from the manufacturing and distribution of a product toxic to the DNA. These vendors will defend themselves vehemently. It is anticipated that part of the defense process will be a denial of the damage done. Recognition that the genetic damage done by the opioids actually places the injured into a separate and distinct subpopulation renders as mute their denials. A DNA test that can withstand a withering assault against the integrity of the its science will be required. Only in this manner can those responsible be held accountable. Artificial Intelligence has the capacity to render as impossible the ability for the pharmaceutical manufacturers and distributors to deny their culpability. What remains to be seen is whether the culpable voluntarily admit their actions and step forward to assist the injured or whether it will require the courts to hold the offenders accountable. Either pathway will depend upon the strength of the genetic science.

1.2). MACHINE LEARNING

Machine learning is a type of Artificial Intelligence. There are three types of machine learning: supervised learning, unsupervised learning, and reinforced learning. For the purposes of establishing two separate and distinct genetic populations and developing a clinical lab test involving levels of DNA methylation, we chose to utilize both unsupervised machine learning and supervised machine learning. We did not see a role for reinforced learning.

1.2.1). UNSUPERVISED MACHINE LEARNING

Unsupervised machine learning is an effective strategy for working with large amounts of data such as epigenetic methylation levels. Untagged data is the input. The machine, through mimicry, utilizes algorithms to build a concise structure of its world and then extract content from this artificial world. The two main methods of unsupervised machine learning are Principal Component Analysis (PCA) and Cluster Analysis. We are specifically using unsupervised machine learning to understand differences between our two data sets: DNA from the opioid naïve (n = 5) and DNA from our opioid dependent (n = 15). If the machine is able to visualize this data as two separate and distinct populations, then this would be the next step in

advancing the epidemiological uniqueness required to define a new disease state and development of a DNA based clinical test. If the machine is not able to visualize the two populations, then resolution of the diagnostic dilemma and development of a clinical lab test may not be feasible. And if the machine cannot visualize the two groups, opioid naïve and opioid dependent, as separate, than neither will the clinician nor the court. But if the machine does see the separation between the two groups, then so must the clinician and the court also see the distinction. If the machine CAN see it, then the clinician and the Court MUST see it. Separating the opioid naïve and the opioid dependent and seeing each group genetically independent is the critical step in ending the Opioid Crisis through the Scientific Process. Unsupervised machine learning can accomplish this task and without prejudice or intent of outcome. Artificial Intelligence is a non biased arbitrator in the matter.

1.2.2). SUPERVISED MACHINE LEARNING

One distinction between unsupervised and supervised machine learning is that while unsupervised machine learning uses untagged data, supervised machine learning uses tagged data. This is part of how supervised machine learning is able to construct algorithms that can learn and then make predictions on data. In this manner, supervised machine learning is able to take our DNA data, now clearly tagged as opioid naïve and opioid dependent and with all associated methylation levels in the data set and build an algorithm that can take a new set of data and predict which of the two known patterns are most alike to the new data: opioid naïve or opioid dependent. This is precisely what we need our clinical test to accomplish. To the best of our knowledge, and except for some bioassays used in cancer screening, this is the first time that Artificial Intelligence has been used in the development of a clinical test involving methylation levels.

2). METHODS

2.1) COLLECTION OF THE SAMPLE

The original plan had been to collect the saliva sample simultaneously with the plasma catecholamine samples and in the medical office setting. But the Zymo DNA collection devices were on back order. When the collection devices were available they were thus mailed to the participants home address. Saliva samples were self collected and returned to the lab at room temperature and utilizing the United States Postal Service. No special transportation parameters were required. While this did result in a fewer number of samples for analysis than had been anticipated, it did show proof of the concept that self collected samples transported with no special requirements and via the USPS were more than adequate for DNA analysis. This was deemed important for the development of a nationwide screening test for the genetic damage done by the opioids.

2.2). SAMPLE STORAGE AND PREP

Once at the lab, the storage and preparation protocols were those in place by EpiGendix. We had no special requests or requirements.

2.3). SAMPLE ANALYSIS

The sample analysis were by the standard protocols established by Epigendx for bisulfate bath methylation analysis. We had no special requests.

2.4). STATISTICAL ANALYSIS

Statistical analyses were performed using R (R version 4.2.1), the program for statistical computing. Descriptive statistics of the DNA methylation data of CpG sites was summarized, comparatively grouped by the Control and Experimental groups. All boxplots, density plots, bar plots, and heatmaps were created using ggplot2 (version 3.3.6). All tables were produced by "kable" in the kableExtra package (version 1.3.4) Spearmen's correlations comparing each CpG site were explored, connecting those relationships with the Promoter and non-Promoter regions of the OPRM gene. Correlations and visualizations were constructed by the "ggcorr" function in GGally (version 2.1.2). Principal Component Analysis (PCA) and Non-Metric Multidimensional Scaling (nMDS) were implemented to visualize group separation of the samples. nMDS was built using the "metaMDS" function in the vegan package (version 2.6-2) specifying for Euclidean distance, and PCA used the "prcomp" function in base stats package. Biplots and scree plots were produced from the "fviz" function in factoextra package (version 1.0.7), as well as ggplot2.

The analysis of DNA methylation percentages of the OPRM gene were evaluated at a CpG site basis, stratified by the two groups of interest: Control and Experiment. Multiple tests for comparison of the groups were analyzed using the non-parametric Wilcoxon- Mann-Whitney (WMW) U-test, also known as the Wilcox Rank Sum Test. The WMW U- test was performed using wilcox_test function in the rstatix package (version 0.7.0), specifying the test as a one-sided, unpaired test. The 16 tests (for the 16 CpG sites) were corrected using the Benjamini-Hochberg correction. Additionally, the Kruskal- Wallace test ("kruskal_test") and one-way ANOVA test ("anova_test") were implemented as weaker alternatives to the WMW U-test, both from the rstatix package.

A PERMANOVA model (non-parametric) was built using the "adonis2" function in the vegan package (version 2.6-2) to investigate the multivariate relationship of the CpG sites by group. Measures of dissimilarity, or distances, were calculated using Euclidean distances. Additionally, a MANOVA model (parametric, unbalanced) was created as an alternative utilizing the "manova" function in the base stats packages, and tested using the "Manova" function in rstatix. The test specified for a type III test for unbalanced design, to account for the unequal sample sizes between groups.

All assumptions for statistical methods were checked using the "mvn" function in the MVN package (version 5.9), and "levene_test" function for equal covariance from rstatix.

3). RESULTS

3.1). HYPERMETHYLATION IN THE OPIOID DEPENDENT

As can be seen in Figure 1, we found multiple sites of hypermethylation in the opioid dependent group versus the opioid naïve group. But we were not simply looking for evidence of hypermethylation due to the opioids. Specifically, we were looking for hypermethylated CpG sites that would be suitable for reliably distinguishing the opioid naive from the opioid dependent and for development of a clinical lab test via Artificial Intelligence. Thus, we were looking for a subset of hypermethylated CpG sites that would be amendable to the requirements of machine learning. Ideally, this would be a population of hypermethylated CpG sites with at least some strong collinearity as this could allow us the opportunity to search for good population separation using Principal Component Analysis. Good population separation was the key to resolving the diagnostic dilemma and development of an effective lab test. The further the graphical difference between the opioid naïve and the opioid dependent, the stronger the scientific evidence.

Figure 1

3.2). 16 CONSISTENTLY HYPERMETHYLATED SITES

16 CpG sites were selected for further evaluation. These 16 sites are seen in Figure 2 and Table 1. Ten sites within the promoter region and 6 non-promoter region sites were selected based upon their consistent hypermethylation between samples in the opioid dependent group. It was noted that these 16 consistently hypermethylated sites included sites of predominantly low methylation within the promoter region and sites of predominantly high methylation located outside of the promoter region. This is consistent with known science. We chose to use this low methylation/high methylation distinction to break the 16 CpG sites down into two groups.

Figure 3 is a bar plot depicting methylation differences between the opioid naïve group and the opioid dependent group in the low methylation sites within the promoter region. Figure 4 is a similar representation but for the six higher methylated sites located outside the promoter region.

Figure 2

Table Table 1

Figure 3

Figure 4

3.3). SPEARMAN'S RANK-BASED CORRELATIONS

3.3.1). COLLINEARITY OF THE TEN CPG SITES WITHIN THE PROMOTER REGION

We next looked at the collinearity within the ten CpG sites in the promoter region. Figure 5 compares the collinearity between our CpG sites within the promoter region of the OPRM1 gene. The Spearman's rank-based correlations between the sites are depicted in each box. Overall, there is relatively strong collinearity between the methylation percentages of our CpG sites within the promoter region. This strong collinearity means that unsupervised machine learning such as Principal Component Analysis could prove to be useful in separating the two populations: the opioid naïve and the opioid dependent. Principal Component Analysis is a widely used, recognized, and accepted form of machine learning. If Principal Component Analysis can visualize the two groups as distinct, then this is a significant step towards establishing the epidemiological uniqueness of the genetically damaged population and determining a proper DNA based clinical test. If Principal Component Analysis cannot recognize the two genetic populations as distinct, then this would be supportive of the mental health diagnosis of Opioid Use Disorder.

Figure 5

3.3.2). COLLINEARITY OF THE SIX CPG SITES OUTSIDE THE PROMOTER REGION

We next looked at the collinearity between our CpG sites located in the non-promoter region of the OPRM1 gene. The Spearman's rank-based correlations between the sites are depicted in each box as seen in Figure 6. Overall, there is relatively weak collinearity between the methylation percentages of our CpG sites outside of the promoter region.

Figure 6

3.4). PRINCIPAL COMPONENT ANALYSIS (PCA, A FORM OF UNSUPERVISED MACHINE LEARNING)

Principal Component Analysis is a dimension reduction technique particularly useful in the setting of a large quantity of variables and a small sample size. As we have 16 CpG sites and 19 samples and with strong collinearity between several variables, PCA could be insightful. PCA is a form of unsupervised machine learning. Table 2 depicts each Principal Component down to a granular level. Each value in the Principal Component represents the contribution (loading) from the corresponding CpG site.

Table 2

3.4.1). IMPORTANCE AND VARIANCE

Table 3 is the cumulative proportion of variance illustrated for each Principle Component. As can be seen, using the first five Principal Components, 86.9% of the total variability of the original 16 CpG sites is maintained. The total variability explained begins leveling off at PC6. We can see this visualized in a scree plot.

Table 3

3.4.2). SCREE PLOT

A scree plot, or elbow plot, visualizes the optimal number of Principal Components to chose. The location in the curve where the line levels off, called the elbow, is considered an adequate cutoff point. Figure 7 is the scree plot from our Principal Component Analysis. This scree plot shows that between 4 and 5 Principal Components is our optimal cutoff. Therefore, 5 Principal Components is the subset of Principal Components in order to retain most of the variation created by the original data, 86.9% to be exact. This is the key information required to build a DNA based clinical test.

Figure 7

3.4.3). BIPLOT GRAPH

A biplot graph uses points and vectors to represent structure. The Principal Component scores are the points and the loading of the samples are the vectors. Figure 8 biplot plots PC1 against PC2. This visualization highlights the separation between the Control Group (opioid naive) and the Experiment Group (opioid dependent) created by the first two PCs. Overall, the Control Group is plotted in the bottom left quadrant indicating that the Controls have PC1 and PC2 values that are smaller and negative. However, the Experiment Group are primarily grouped from the middle to the right, meaning that the Experiment Group has higher values than the Control Group. This has been the goal of our efforts to this point. We asked the machine to give us the scientific evidence that the two groups were separate and independent from each other. This goal has been achieved. This is strong scientific evidence in the resolution of the diagnostic dilemma. The fact that the machine sees the two genetic populations as separate and distinct is evidence supporting the diagnosis of genetic damage and evidence undermining the diagnosis of Opioid Use Disorder. This separation of the opioid naïve and opioid dependent based upon epigenetic data and establishing the proper diagnosis may well be one of the most important contributions to humankind yet by Artificial Intelligence.

Figure 8

3.5). NON-METRIC DIMENSIONAL REDUCTION SCALING (NMDS, A TYPE OF UNSUPERVISED MACHINE LEARNING)

Like Principal Component Analysis (PCA), Non-Metric Dimensional Reduction Scaling (NMDS)is a dimensional reduction technique. NMDS optimizes stress, which are values equivalent to the difference in distance between the reduced dimension and the full dimension (the original data). Optimizing the stress values means that the algorithm will try to minimize the stress and therefore maximize the similarities between the reduced and full dimensions. NMDS differs from PCA as NMDS relies upon these calculated stress values for orientation. PCA relies upon the loadings calculated by eigenvalues/eigenvectors. Figure 9 is the NMDS plot of MDS1 against MDS2. The distances calculated are measured in Euclidean. The data was transformed using Hellinger's square root method. We specifically underwent NMDS as a means of comparing the outcome of the NMDS to the outcome from the PCA. We felt it was important that we understood whether or not the two processes found agreement.

NMDS produced a very similar plot/values as PCA. Just the spread of each group is slightly different. The Control Group is again predominantly on the negative side of the x-axis. The Experimental Group is again predominantly on the middle/positive side of the x-axis. The outliers are noted, but not too concerning given the sample size. Overall, this is a reassurance that the opioid naïve and the opioid dependent are separate entities genetically and can be identified as unique and different. In other words, NMDS, another widely recognized and accepted form of machine learning, is found to be in strong agreement with the PCA. In our opinion, the diagnostic dilemma is now settled. Artificial Intelligence has visualized the two populations as separate and distinct and with replication of the findings. The mental health diagnosis of Opioid Use Disorder is debunked. But just to be complete, we will run one more deep machine learning analysis, PERMANOVA Analysis. But first we will correct our p-values.

Figure 9

3.6). CORRECTING THE P-VALUES

This data undermines an entire industry built upon a false mental health diagnosis. Billions of dollars are involved. Therefore, push back against this data is an expectation. As we have seen above, both the Principal Component Analysis and the Non-Metric Dimensional Reduction Scale visualized two separate and distinct populations in the data: the opioid naive and the opioid dependent. In order to support this position of two separate populations, we present now the p-values corrected in various methods. As always, alpha = 0.05.

3.6.1 MANN-WHITNEY U-TEST (NON-PARAMETRIC)

The Mann-Whitney U-Test, also known as the Wilcoxon Rank Sum Test, is a specific version of the Kruskal-Wallace Test but intended for exactly two groups. The Null Hypothesis is that the two groups come from the same population. This is considered the most powerful and applicable test to use on our dataset. As can be seen in Table 4, using alpha = 0.05 and correcting the p-values, 11 of the 16 CpG sites (bolded) are significantly different between the Control (opioid naive) and Experimental (opioid dependent) groups.

Table 4

3.6.2). KRUSKAL-WALLACE TEST (NON-PARAMETRIC)

The Kruskal-Wallace Test is the non-parametric alternative to a one-way ANOVA (Analysis of Variance) and is a broader version of the Mann-Whitney U-Test. Instead of testing group means, the Kruskal-Wallace Test analyzes for significant differences between the mean ranks of the groups. The Null Hypothesis is that the

two groups come from the same population. As can be seen in Table 5, using alpha = 0.05 and correcting the p-values, 7 of the 16 CpG sites (bolded) are significantly different between the Control (opioid naive) and the Experimental (opioid dependent) groups.

Table 5

3.6.3). ANOVA TEST (PARAMETRIC) (ANALYSIS OF VARIANCE)

The one-way ANOVA Test is a parametric approach to compare two or more groups. The Null Hypothesis of the ANOVA Test is that there is no difference among group means. As can be seen in Table 6, using alpha = 0.05, and correcting the p-values, 5 of the 16 CpG sites (bolded) are significantly different between the Control (opioid naive) and the Experimental (opioid dependent) groups.

Table 6

3.7). PERMANOVA ANALYSIS (PERMUTATIONAL MULTIVARIATE ANALYSIS OF VARIANCE)

PERMANOVA is used to test whether groups of objects are significantly different. The test statistic is the pseudo-F-ratio. The Null Hypothesis for the PERMANOVA is that the centroids and dispersions of the groups as defined by measured space are equivalent for all groups. Our PERMANOVA model was created using the 16 CpG sites as the dependent variables and Group as the independent variable. As can be seen in Table 7, using alpha = 0.05, and with $P < 0.0065$, we should reject the Null Hypothesis. This means that there is some difference between the centroids/dispersions of each group. This finding supports the group separation seen by both PCA Biplot (figure 8) and NMDS Plot (figure 9). We are now at a point where three of the most widely used and accepted methods of Artificial Intelligence, deep machine learning, are in agreement. The two genetic populations are separate and distinct. From this point forward, the mental health diagnosis of Opioid Use Disorder is no longer appropriate in the setting of an individual with evidence of hypermethylation in the promoter region of the OPRM1 gene. Furthermore, to make the diagnosis of Opioid Use Disorder and without proper evaluation of the individual's DNA is considered an act of malpractice.

Table 7

3.8). THE TWO GROUPS BEHAVE DIFFERENTLY

The PCA, the NMDS, and the PERMANOVA are in agreement. The corrected p-values are significant. The machine is an independent arbitrator. The machine is not a part of the vast medical/pharmaceutical industrial complex. The machine does not have a stake in the outcome. The machine does not have "opinions". And the machine clearly sees two separate and distinct populations: the opioid naive and the opioid dependent. This will forever shatter the mental health diagnosis of Opioid Use Disorder. Artificial Intelligence has led the way. The opioid dependent do not have a mental health condition. The opioid dependent have damaged DNA. From this point forward, and thanks to the clarity provided by Artificial Intelligence, the proper diagnosis of genetic damage will be recognized and creating the final algorithms for the clinical test is a straightforward undertaking.

And, most importantly, when we bring in the data from our companion article entitled Part 1: Behaving Differently we can now see clearly that not only are there two populations, but that they behave differently. And that is the point. The two populations behave differently. Under identical conditions of opioid abstinence, the opioid naive show no change in behavior. However, under identical conditions of opioid abstinence, the hypermethylated group demonstrates a marked change in behavior, Table 8. The group

identified by Artificial Intelligence as hypermethylated, experience an elevated Autonomic Dysfunction Scale during opioid abstinence. The group identified by Artificial Intelligence as hypermethylated, experience an elevated Opioid Craving Scale during opioid abstinence. The group identified by Artificial Intelligence as hypermethylated, experience catecholamine toxicity during opioid abstinence. And, again from our first companion article, we know catecholamine toxicity puts the individual at risk of a catastrophic cardiovascular event. Thus, the group identified by Artificial Intelligence as hypermethylated, experience a higher risk of a catastrophic cardiovascular event during opioid abstinence. The two populations are separate and distinct genetically and the two populations behave differently in the setting of opioid abstinence. The mental health diagnosis of Opioid Use Disorder is an inappropriate diagnosis. The proper diagnosis is Autonomic Dysfunction due to genetic damage secondary to repetitive opioid exposure. Artificial Intelligence has played a significant role in the recognition of a new disease state. Artificial Intelligence has just served humankind in one of its most important roles to date.

Table 8

3.9). THE FINAL ALGORITHMS FOR THE CLINICAL TEST FOR DNA HYPERMETHYLATION DUE TO REPETITIVE OPIOID EXPOSURE

We are continuing to analyze DNA from those damaged by the opioids and contrasted against those who are opioid naive. We are not ready at this point to publish the findings as we would like to run a larger dataset prior to publication. That stated, the two other accompanying articles were ready for publication. It is our plan to publish more about the final algorithms at a future date.

6). DISCUSSION

In May of 2015, Dr. David Nutt et al published the article The Dopamine Theory of Addiction: 40 Years of Highs and Lows. Dr. Nutt was addressing the mental health community of researchers and academicians. In the article, Dr. Nutt stated: "The apparent rush to publish that any given pleasure-inducing drug or behavior can induce dopamine release reflects one of the more worrying and pervasive aspects of science today - the pre-eminence given to reporting "positive" data in support of currently influential theories. There is a concern that the classic Popperian approach to science, namely refuting hypothesis, may be lost in the desire to publish papers that "prove" the theory and are then well cited but often not replicated". And well that Dr. Nutt spoke up. Dr. Nutt is an internationally recognized authority on the subject and served as drug czar under British Prime Minister Tony Blair. Dr. Nutt is also a co-author on the two studies cited in Part One of this trilogy, Daglish et al (2008) and Watson et al (2014). It was these two studies, utilizing the superior technology of the PET Scan, that showed and replicated that there was no surge in brain dopamine in humans and in response to the administration of an opioid. In other words, Dr. Nutt and his colleagues had refuted the Dopamine Theory of Addiction as it pertains to the opioids. To continue to advocate for the Dopamine Theory of Addiction in regards to the opioids after the theory had been refuted is contrary to classic Popperian Science and contrary to the Falsification Principle as put forth by Karl Popper himself. In other words, it is unscientific to continue to advocate for the Dopamine Theory of Addiction in regards to the opioids. But it was the only theory that the mental health community had in regards to the opioids. And this is the issue that Dr. Nutt was addressing with the statement quoted above.

The reply from the addiction community was swift and united. In January of 2016, a brief 8 months after the publication by Nutt et al, an article appeared in the New England Journal of Medicine titled Neurobiologic Advances from the Brain Disease Model of Addiction . The article was co-authored by Dr. Nora Volkow, current Executive Director at the National Institute for Drug Abuse (NIDA), Dr. George Koob, former Chair of Neurobiology of Addictive Disorders at the Scripps Research Institute, and Dr. A. Thomas

McLellan, current Board Member at Indivior (a pharmaceutical manufacturing company with the indication to manufacture under Opioid Use Disorder). These were individuals and institutions highly vested in the mental health diagnosis of Opioid Use Disorder. In the article and in reference to the dopamine theory, it is stated "All known addictive drugs activate reward regions in the brain by causing sharp increases in the release of dopamine". The statement did not exempt the opioids. The entire article failed to even mention the conflicting work by an internationally recognized team. Volkow et al give three citations in support of the broad statement. None of the three mention the opioids. And if this was not enough, in 2019, Dr. Yngvlid Olsen, then Vice-President of the American Society of Addiction Medicine (ASAM), and Dr. Joshua Sharfstein, then Principal Deputy Commissioner of the U.S. Food and Drug Administration co-authored a book entitled The Opioid Epidemic What Everyone Needs to Know. And in regards to dopamine and the opioids, the following statement is made: "Deep in the reward center of the brain of someone with opioid addiction, an opioid causes - at least at first - an enormous release of the neurotransmitter dopamine." That these statements could be made after the findings of Daglish et al in 2008 (no increase in brain dopamine in response to the administration of an opioid) and Watson et al in 2014 (no increase in brain dopamine in response to the administration of an opioid) is perhaps exactly what Dr. Nutt had been warning. Science had not supported their position. Had they chosen opinion over science? Once one is relieved of the burden imposed by the Scientific Method, one is free to say about anything.

We had no interest in entering this scientific debate, particularly as some had abandoned the principles of science but continued to participate in the dialogue. And we did not want to repeat the experience of Daglish, Watson, and Nutt of publishing our results only to have them ignored as an inconvenience. We lay out these actions by powerful members of the mental health industry as a groundwork to understand why we began to consider the use of Artificial Intelligence. This abandonment of science by some who seemed to have a stake in the outcome of the debate led us to the use of the machine, Artificial Intelligence. The machine is void of opinion. The machine does not have a stake in the outcome. The machine is linked to neither a pharmaceutical manufacturer nor is the Executive Director of any organization. The machine is incapable of abandoning science. The machine is a perfect arbitrator. And we simply said to the machine, "Do you see one genetic population, thus supporting a mental health diagnosis, or do you see two genetic populations, thus supporting a genetic damage diagnosis"? And the machine clearly visualized two separate and distinct genetic populations. And this visualization of the two separate and distinct populations was replicable. A new disease state has been defined. The inappropriate diagnosis of Opioid Use Disorder is debunked. The proper diagnosis of autonomic dysfunction due to the genetic damage of the opioids is established. The goals of this article have been met.

This does not mean that the noise will stop. Most likely, the noise from the mental health industry will only increase in volume, intensity, and destructiveness. But while they will continue to argue, their arguments are now futile. But while they will continue to advocate, their advocations are now futile. But while they will continue to deny, their denials are now futile. The issue is settled, partially due to the strength and capabilities of Artificial Intelligence. This population has had their DNA damaged by the opioids. When they attempt opioid abstinence, they encounter autonomic dysfunction and are secondarily craving opioids as a means to escape the agony of the autonomic dysfunction. We now have answers. The art has been advanced.

CONCLUSION

The previous article, Part One: Behaving Differently had already established that the two populations behaved differently in the identical scenario of opioid abstinence. In this article, Part Two: Two Separate and Distinct Populations, we have used Artificial Intelligence to help us establish that the two populations are genetically separate and distinct. These two criteria, a separate and distinct population behaving differently

when compared to the controls, now define this separate population as meeting the criteria as a disease state. This raises the next important question: What to do? We will explore this question in detail in the third and final installment in this trilogy. In this next article, we will formally define our new disease state and set forth a proper treatment plan.