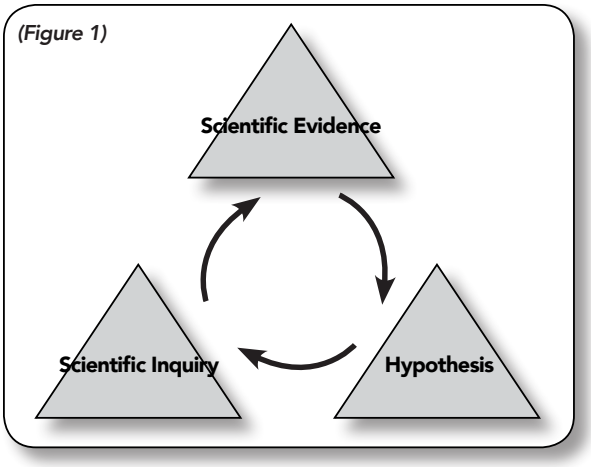
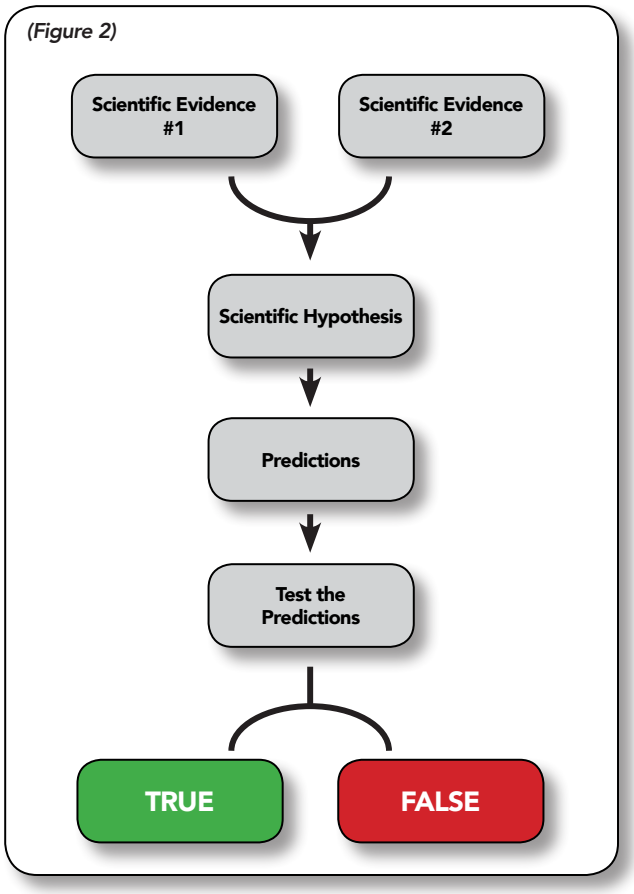
TWO ACCURATE PREDICTIONS BY THE SMITH HYPOTHESIS



The modern Scientific Method has been in place for generations. At it's foundation, the modern Scientific Method relies upon the formation of a hypothesis to explain observed scientific evidence. This hypothesis should then lead to predictions. These predictions can then be subjected to a scientific inquiry. In this manner, a hypothesis can either be strengthened, modified, or discarded. When predictions from a hypothesis are found to be accurate, the hypothesis is considered strengthened. ***(Figure 1)***



The purpose of this article is four fold:

1). To introduce the Smith Hypothesis.

2). To introduce the two fields of scientific evidence that led to the formation of the Smith Hypothesis.

3). To introduce the two predictions made by the Smith Hypothesis.

4). To present the scientific evidence that supports the two predictions by the Smith Hypothesis.

It is important to note that it was not one body of scientific evidence that led to the development of the Smith Hypothesis. Instead, the Smith Hypothesis was the result of combining two separate and seemingly unrelated bodies of scientific evidence:

1). Scientific evidence of a type of toxicity to the DNA known as methylation and as a result of exposure to the opioids.

2). Scientific evidence for the occurrence of autonomic dysfunction seen in so called, "opioid withdrawal."

By combining these two seemingly unrelated bodies of scientific evidence into one hypothesis, a clearer picture of the opioid landscape begins to emerge. The Smith Hypothesis was formed. Predictions were made from the hypothesis. And we now have scientific evidence supporting the accuracy of these two predictions.

Scientific Evidence For a Dysfunction in the Autonomic Nervous System Due to the Opioids

As stated, the Smith Hypothesis emerged from combining two seemingly unrelated bodies of scientific evidence. The first body of scientific evidence is a series of articles and studies that relate to a dysfunction within the Autonomic Nervous System and in those study subjects who were experiencing what is commonly referred to as "opioid withdrawal". This body of scientific evidence is broad and with several well done studies seeming to replicate and support the findings of other, previous studies. These studies expand across a substantial timeframe and begin well over 50 years ago. However, this line of inquiry seems to end abruptly in the 1990s. One could well ask what role stopping this important line of inquiry had upon the depth and duration of the ensuing Opioid Crisis. One could also ask what decisions led to a landscape wherein an important field of scientific inquiry was allowed to wither.

Scientific Evidence For Damage to the DNA Known as Methylation Due to the Opioids

As stated, the Smith Hypothesis emerged from the combining of two seemingly unrelated bodies of scientific evidence. As noted above, the first body of scientific evidence referenced a series of well done articles and studies in relation to a dysfunction within the Autonomic Nervous System seen during so called "opioid withdrawal". As further noted, this line of scientific inquiry mostly ceased during the 1990s.

However, a second and seemingly unrelated body of scientific evidence, began to emerge beginning in 2009 and continuing. This is a series of well done studies in regards to the methylation found to be occurring in the DNA of humans whose sole uniting common feature was an exposure to the opioids. Methylation is known as a form of toxicity, damage to the DNA. This toxicity to the DNA known as methylation results in areas of the DNA being turned off, a process called Gene Silencing. In the case of the opioids, this Gene Silencing is seen to be occurring exclusively in a highly sensitive area of the DNA known as the promoter region. Specifically, this methylation within the promoter region was found to be occurring within the gene that encodes for the primary opioid receptor. This primary opioid receptor is known as the (mu)-opioid receptor. Methylation within the promoter region of a gene has the potential for profound effects upon the individual. Researchers anticipated a down regulation of the number of (mu)-opioid receptors. A down regulation of the (mu)-opioid receptor would have resulted in fewer (mu)-opioid receptors to have been found within the body. But a reduction in the number of (mu)-opioid receptors has yet to be borne out by the scientific data.

This lack of a discoverable down regulation in the population of (mu)-opioid receptors has led to a new scientific possibility called "Partial Gene Silencing". Partial Gene Silencing is not currently an established reality within the scientific community. Partial Gene Silencing is only being introduced by and within the Smith Hypothesis. In simplified terms, Partial Gene Silencing holds forth that a normal number of (mu)-opioid receptors is being produced within the body of those whom have been methylated. However, these (mu)-opioid receptors are abnormal and unable to perform the normal duties of a (mu)-opioid receptor. It is further postulated by the Smith Hypothesis that at least a partial function of the (mu)-opioid receptor is a regulation of the balance within the Autonomic Nervous System. If this postulation by the Smith Hypothesis is subsequently supported by further scientific evidence, then this begins to explain why individuals apparently lose control of the their Autonomic Nervous System during so called "opioid withdrawal". This would further begin an understanding of why control of the Autonomic Nervous System is at least partially established with the consumption of either full or partial agonist opioids.

THE SMITH HYPOTHESIS

100% of the deaths associated with the Opioid Crisis were premature and mostly preventable deaths.

Methylation of certain CpG islands within the promoter region of the OPRM1 gene, as seen in response to the exposure to the opioids, results in gene silencing. This gene silencing is not producing a drop in the population of the mu-opioid receptor. Rather, this gene silencing results in the formation of an abnormal mu-opioid receptor. This production of an abnormal mu-opioid receptor is due to a process we are calling partial gene silencing - the receptor was produced but it was an abnormal receptor. This abnormal mu-opioid receptor is no longer able to maintain balance and homeostasis within the Autonomic Nervous System when Opioid Abstinence is attempted. This dysfunction within the Autonomic Nervous System results in a true Neuroendocrine Emergency known as Autonomic Dysfunction. This Autonomic Dysfunction is reflected in the abnormal activity in both branches of the Autonomic Nervous System, the Sympathetic Nervous System and the Parasympathetic Nervous System. Autonomic dysfunction, and the Catecholamine Surge that results, is a condition of extreme duress and cannot long be endured by the human body. The full agonist opioids offer a partial and temporary relief. But this partial and temporary relief comes with the associated risk of the full agonist opioids. The partial agonist, Buprenorphine, is able to maintain a more complete and longer lasting relief from the autonomic dysfunction but, due to the Ceiling Effect of Buprenorphine, at a higher level of safety. Untreated, there is concern that autonomic dysfunction could be a risk factor for the development of Opioid Induced Adrenal Insufficiency. In addition, a Catecholamine Surge is known to be associated with Cardiomyocyte Necroptosis. Therefore, cardiac related death rates would be expected to be increased dramatically in any vulnerable populations which also experienced a simultaneous increase in opioid overdose deaths. These cardiac deaths would mistakenly be attributed to a non opioid related etiology.

At this point in time, we will now introduce the Smith Hypothesis. As noted above, the Smith Hypothesis is a result of the combining of two seemingly unrelated bodies of scientific evidence. The combination of the body of science relating to autonomic dysfunction seen in so called "opioid withdrawal" and the body of science relating to the methylation within the promoter region of the OPRM1 gene due to an exposure to the opioids. This combining of the two seemingly unrelated bodies of scientific evidence has resulted in the formation of the Smith Hypothesis.

While some of the science within the Smith Hypothesis is admittedly complex, the Smith Hypothesis itself is an easy concept to understand. All that the Smith Hypothesis is saying is that damage to the body due to an exposure to the opioids results in a dysfunction within a major organ system within the body. There is no conceptual component that is complex. The opioids damage the DNA of a fundamental and important component of the body, namely the main opioid receptor. Once damaged, this receptor is unable to complete all it's tasks. Apparently, one such task negatively impacted is maintaining balance and control within the Autonomic Nervous System. This concept of a poisoning to the body resulting in an abnormal function within the body is as simple as cause and effect. It's just that simple- cause and effect. The opioids poison the DNA. This poisoning of DNA results in a dysfunction within the Autonomic Nervous System. It's just that simple, cause and effect.

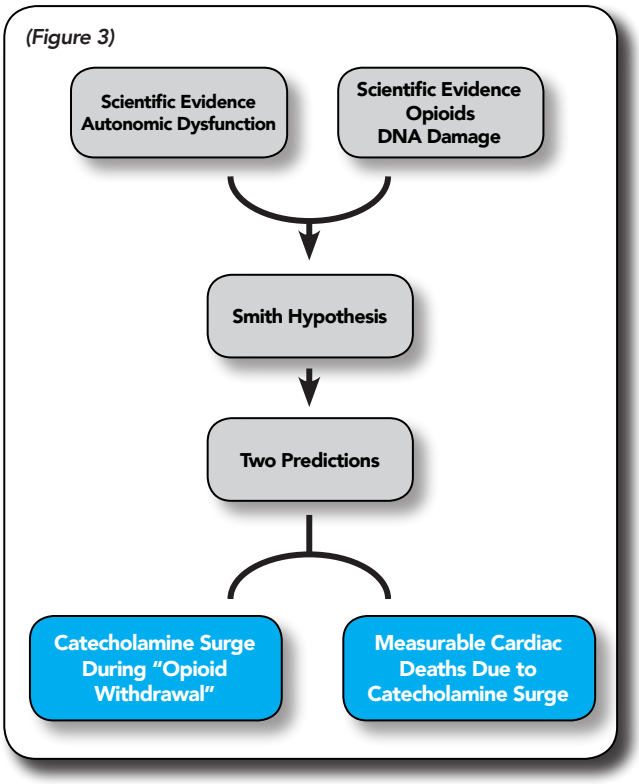
The Smith Hypothesis is exactly that- it is a hypothesis. The purpose of a hypothesis is a proposed explanation

of observations. In this instance, the hypothesis is a proposed explanation for the autonomic dysfunction seen during so-called "opioid withdrawal". The proposed explanation is that damage to the DNA of the (mu)-opioid receptor results in a dysfunctional receptor. This dysfunctional receptor is no longer able to maintain balance and homeostasis of the Autonomic Nervous System when opioid abstinence is attempted. This means that when people who have taken opioids long enough to have accumulated some threshold of damaged receptors, when these individuals stop taking the opioids, they lose control of their internal organs. The human body cannot endure this loss of control of the internal organs. The victim is driven into a desperate state. For most victims, the only way out of this horrible condition is to take an often illegal opioid.

The question then arises, how strong is this proposed hypothesis? And by what criteria is the strength of a hypothesis measured? The answer is that a hypothesis is measured by it's ability to make predictions that can then be evaluated. And the Smith Hypothesis is no different. Part of the strength of the Smith Hypothesis is in the ability to explain the observations with clarity. And part of the strength of the Smith Hypothesis is the ability to make predictions that can then be measured. So, what predictions arise from the Smith Hypothesis? There are a number of strong predictions that arise from the Smith Hypothesis. We present here two of the strong predictions from the Smith Hypothesis. And then we will present data demonstrating the accuracy of both predictions arising from the Smith Hypothesis. The more accurate the predictions, the stronger the hypothesis.

The first prediction arising from the Smith Hypothesis is that a Catecholamine Surge will be detectable during a period of autonomic dysfunction, what was previously referred to as "opioid withdrawal". It is noted that this autonomic dysfunction is a loss of control of both branches of the Autonomic Nervous System, the Sympathetic and Parasympathetic Nervous Systems. Therefore, since this is more than simply Sympathetic Nervous System toxicity, in other words, the Parasympathetic Nervous System is also involved, this predicted Catecholamine Surge will be intermittent. This should be a relatively straightforward prediction for evaluation. Simply allow a number of opioid impacted individuals to stop taking any opioid and thus go into a state of opioid abstinence autonomic dysfunction. Then, simply draw blood levels for routine Catecholamine evaluation. And this is exactly how the following data was obtained. Under IRB approval and Informed Consent, blood levels for Catecholamines were obtained during a period of autonomic dysfunction due simply to an opioid abstinence. And the results are shocking.

(Figure 3)



And this leads into the second prediction arising from the Smith Hypothesis. Catecholamine Surge is not a safe or benign state. Catecholamine Surge is strongly associated with heart complications. Specifically, Catecholamine Surge is associated with Takotsubo Cardiomyopathy and, more deadly, with cardiomyocyte necroptosis. And let's be crystal clear at this point. This second prediction from the Smith Hypothesis is that a significant and measurable number of people are suffering with and needlessly dying from cardiac complications due to the Catecholamine toxicity. This would mean that the overall death rate from this Opioid Crisis is being consistently underestimated. Obviously, the presence of a Catecholamine Surge would negate the mental health diagnosis of "Opioid Addiction". A mental health disorder cannot

be diagnosed in the setting of a true neuroendocrine emergency such as autonomic dysfunction and with associated Catecholamine toxicity. The implications would be that a large number of individuals have suffered and even died premature and preventable deaths due to a failure to recognize the underlying etiology and to apply a proper diagnosis and treatment.

FIRST PREDICTION - A DETECTABLE CATECHOLAMINE SURGE DURING SO-CALLED "OPIOID WITHDRAWAL"

The current in vogue diagnosis for the people suffering from this Opioid Crisis is a mental health diagnosis known as Opioid Use Disorder. Since Opioid Use Disorder is a mental health diagnosis, it can be found in the current DSM-5 (Diagnostic Statistical Manual of Mental Disorders, 5th edition). And that's exactly the point. Opioid Use Disorder (OUD) is found exclusively in the manual of mental disorders. Opioid Use Disorder was the result of combining two previous diagnoses in DSM-4 (Opioid Dependence and Opioid Abuse), into one diagnosis in DSM-5 (Opioid Use Disorder). But the point remains, these are all mental health diagnoses. How do we know that these are all mental health diagnoses? Because they are found exclusively in the manual of mental health disorders known as the Diagnostic Statistical Manual of Mental Health Disorders.

Now that we are clear that Opioid Use Disorder is a mental health diagnosis, let's spend a moment on the fundamentals of medicine. Why spend time on the fundamentals of medicine? Because this is where the error occurred. The error in this Opioid Crisis was a fundamental error in the practice of medicine. And here is a fundamental of medicine - before a mental health diagnosis can be made, all physiologic pathology must first be excluded. It's just common sense. Before a mental health diagnosis can be made, it is imperative that all physiological abnormalities have been excluded. The process by which all physiological abnormalities are excluded always begins with a physical examination and proper lab testing. Ignoring for now that several symptoms of so-called "opioid withdrawal" are simply impossible to simultaneously co-exist (tachycardia, diaphoresis, and excessive yawning), the lab testing itself will tell us the story. The Smith Hypothesis predicts a detectable Catecholamine Surge will be found intermittently during so-called "opioid withdrawal". Thus a study protocol was submitted and obtained IRB approval. And the results are simply overwhelming. 13 out of 15 participants had at least one of the three Catecholamines elevated. 10 out of 15 participants met the criteria for a Catecholamine Surge.

PAR-TICIPANT	Catecholimine		
NUMBER	Nor-pinephrine	EPINERPRI	DOPAMINE
100	279	53	<30
200	**982**	55	39
300	**1859**	15	**109**
400	**971**	<15	40
500	356	**70**	**63**
600	219	36	<30
700	564	**153**	**58**
800	**992**	**154**	35
900	444	**134**	<30
1000	581	26	**89**
1100	380	**88**	<30
1200	529	**65**	<30
1300	**1959**	107	44
1400	586	**87**	**50**
1500	835	15	**67**

(Figure 4)

These results ***(Figure 4)*** were so profound that an emergency halt to the study was required by the rules set forth by HHS. The phrase "unanticipated problems involving risk to subjects or others" is found but not defined in the HHS regulations at 45 CFR part 46. Unanticipated problems include any incident that meets all of the

following criteria:

1). The incident was unexpected in terms of nature severity or frequency.

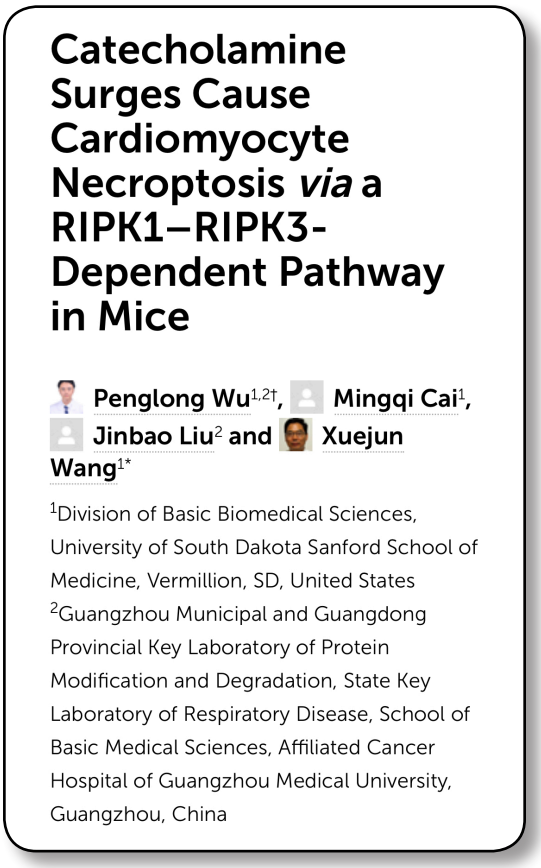
2). The incident was related or possibly related to participation in the research.

3). The incident suggest that the research places subjects at a greater risk of harm including physical, psychological, economic, or social harm than was previously known or recognized.

While the Smith Hypothesis PREDICTED a Catecholamine Surge in so-called "opioid withdrawal," this was not a known scientific fact. As soon as it became apparent that, in fact, the Smith Hypothesis was correct and that a dangerous and life threatening Catecholamine Surge was a profound component of so-called "opioid withdrawal" the regulations set forth by HHS are clear. The research, while groundbreaking and important, must be subjected to an immediate emergency halt of the research. May this emergency halt stand in testimony to the severity of the discovered Catecholamine Surge and to the accuracy of the Smith Hypothesis. The question as to whether or not the Opioid Crisis was driven by a mental health disorder or not has been forever answered. The Opioid Crisis was not driven by a mental health disorder. The Opioid Crisis was driven by a true Neuroendocrine Emergency and with associated deadly Catecholamine Surge. Millions of people had been falsely labeled with an improper diagnosis. Millions of people had been denied a proper treatment. Hundreds of thousands of people died a needless and preventable death. And all because the fundamentals of medicine were ignored.

SECOND PREDICTION - A DETECTABLE INCREASE IN CORONARY ARTERY DISEASE DEATHS IN ANY POPULATION SIMULTANEOUSLY EXPERIENCING AN INCREASE IN OPIOID OVERDOSE DEATHS

We have already seen that the first prediction as a result of the Smith Hypothesis was not only accurate but was so profoundly accurate that the emergency halt measures by HHS for a clinical trial were triggered. We now move onto the second prediction as a result of the Smith Hypothesis - a detectable increase in Coronary Artery Disease deaths in any population simultaneously experiencing an increase in opioid overdose deaths. In other words, individuals who find themselves without an accurate diagnosis and without proper treatment, when experiencing so-called "opioid withdrawal" are left with a dilemma- risk of death by a fentanyl overdose or risk of death by a heart attack. And the Smith Hypothesis is saying that these Catecholamine Surge induced heart attacks are so common and prevalent that these death by heart attacks can be easily measured in any population experiencing a surge in opioid overdose deaths. It is noted that the medical examiner would not note that the specific and individual Coronary Artery Disease death would be opioid related. The simple explanation for this confusion is that no opioids would be found in the body. It was a lack of opioids and the ensuing autonomic dysfunction that led to the heart damage in the first place.



Catecholamine Surges Cause Cardiomyocyte Necroptosis *via* a RIPK1–RIPK3-Dependent Pathway in Mice

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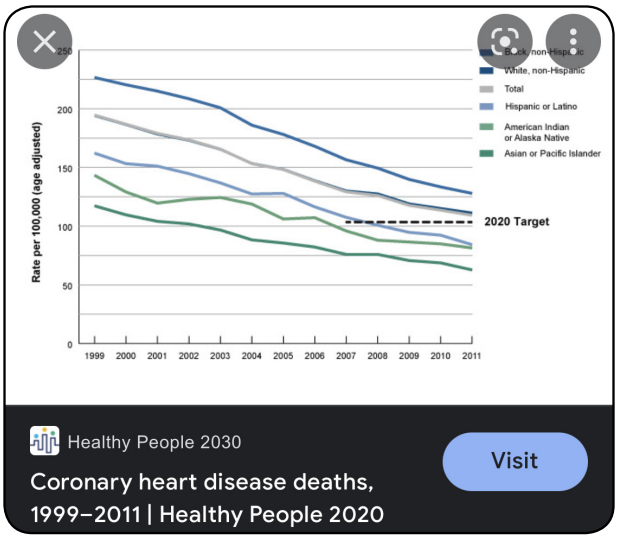
(Figure 5)

The logic here is simple and straightforward. Now that we know that a Catecholamine Surge is a component of the loss

of control of the internal organs during what was previously known as so-called "opioid withdrawal", we only need to recognize that a Catecholamine Surge is associated with cardiomyocyte necroptosis. Cardiomyocyte necroptosis is what the layperson would know as a heart attack. This is an admittedly oversimplification of the science, but we will allow the oversimplification for now. And let's be crystal clear here, we are using as our yardstick for the accuracy of the Smith Hypothesis the endpoint of death by cardiomyocyte necroptosis. This is the most stringent endpoint possible- death by what the layperson would know as a heart attack. The cited article by Wu et al is titled "CATECHOLAMINE SURGE CAUSES CARDIOMYOCYTE NECROPTOSIS" ***(Figure 5).*** There is more to the title. But that's all the knowledge that is required for now. Note the title includes the most powerful language of "CAUSES CARDIOMYOCYTE NECROPTOSIS." The title doesn't use the words "is associated with". No, the title is very explicit- CAUSES. Cause is a powerful word in the scientific community.

Next, let's just take a quick look at the nature of Coronary Artery Disease deaths ***(Figure 6).*** While the overall trend is noted to be a downward trend, what we would like for the reader to note is that surges in Coronary Artery Disease deaths (CAD deaths) simply is not a part of the landscape. Not a single surge in the death rate can be discerned from this multi year graph. Therefore, practically any surge in CAD deaths would beg for an explanation.



(Figure 6)

And, now that we have a deeper understanding of the how and why the Smith Hypothesis is predicting a detectable increase in Coronary Artery Disease deaths in any population simultaneously experiencing a surge in deaths due to opioid overdose, let's now turn our attention to data recently obtained within the homeless population in Los Angeles County, California ***(Figure 7)***. This specific data is year over year epidemiological mortality rates from 2020 to 2021. We first see that year over year, all deaths rose from 1271 to 1988, an enormous increase of 56%. And we further see that this enormous increase in the death rate among the homeless population in LA County is driven predominantly by a 78% surge in drug overdose deaths, predominantly opioids, predominantly fentanyl. But right beneath the 78% surge in overdose deaths, we see exactly what the Smith Hypothesis predicted that we would see - namely a 29% surge in Coronary Artery Disease deaths. The second prediction from the Smith Hypothesis has gained scientific support.

Table 1 - **Number and Characteristics of LA County Deaths among PEH, 12 Months Pre- and Post-pandemic Onset**

Characteristic	Pre-Pandemic Numbers 4/01/19-3/31/20	Post-Pandemic Numbers 4/1/20-3/31/21	Absolute Increase	% Increase
All deaths	1271	1988	717	56%
	Gender			
Male	1037	1618	581	56%
Female	233	370	137	59%
	Age			
18-29	85	175	90	106%
30-49	373	633	260	70%
50-64	585	842	257	44%
65+	228	338	110	48%
	Race/Ethnicity			
Black/African American	325	515	190	58%
Asian	16	34	18	113%
Hispanic/Latinx	486	820	334	69%
White	426	592	166	39%
Other[1]	18	27	9	50%
	Cause of Death			
Drug Overdose	402	715	313	78%
Coronary Heart Disease	239	309	70	29%
COVID-19	0	179	179	NA
Traffic Injury	113	150	37	33%
Homicide	70	104	34	49%
Suicide	55	64	9	16%
Other Unintentional Injuries	54	57	3	6%

[1] Includes American Indian/Alaska Native, Native Hawaiian/Pacific Islander, multiracial, and refused/unknown

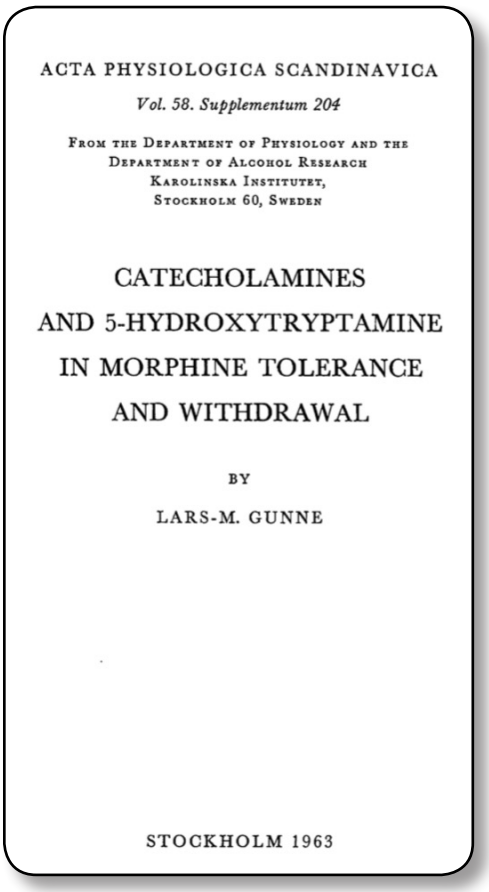
(Figure 7)

Review of World Literature

excerpt from

"THE SCIENTIFIC EVIDENCE, SMITH HYPOTHESIS, & PREDICTIONS"

SCIENTIFIC EVIDENCE FOR DYSFUNCTION WITHIN THE AUTONOMIC NERVOUS SYSTEM AND DUE TO OPIOID DEPENDENCY



ACTA PHYSIOLOGICA SCANDINAVICA

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FROM THE DEPARTMENT OF PHYSIOLOGY AND THE DEPARTMENT OF ALCOHOL RESEARCH KAROLINSKA INSTITUTET, STOCKHOLM 60, SWEDEN

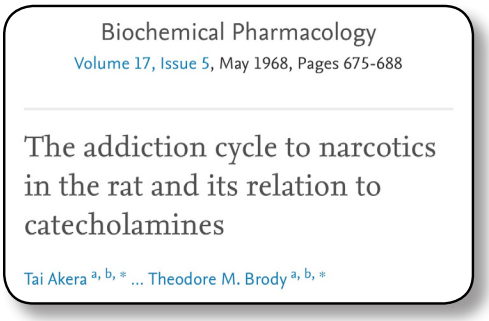
CATECHOLAMINES AND 5-HYDROXYTRYPTAMINE IN MORPHINE TOLERANCE AND WITHDRAWAL

BY

LARS-M. GUNNE

STOCKHOLM 1963

(Figure 6)



Biochemical Pharmacology

Volume 17, Issue 5, May 1968, Pages 675-688

The addiction cycle to narcotics in the rat and its relation to catecholamines

Tai Akera [a, b, *] ... Theodore M. Brody [a, b, *]

(Figure 7)

While there were some studies as early as the 1950s, we have chosen to begin with the study by Gunne (1963). ***(Figure 6)***:

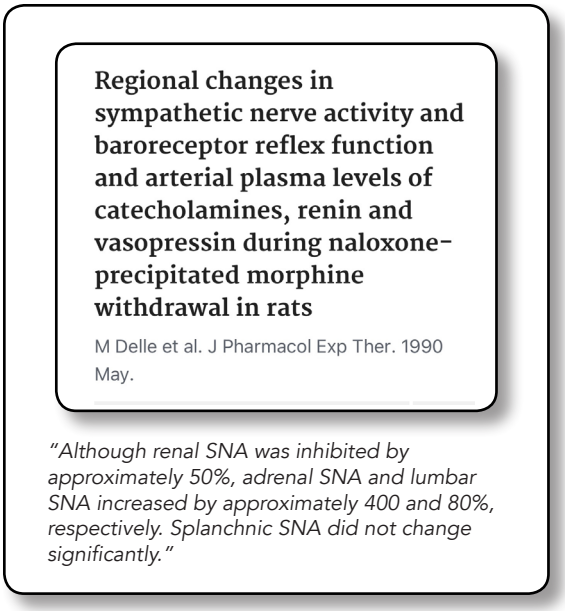
"The content of adrenaline (Epi) in adrenal glands was depleted in chronic morphine-treated rats which experienced withdrawal symptoms 48 hr after abrupt morphine withdrawal" (Gunne, 1963).

We believe that we are interpreting this quote as the author intended. Gunne was making the observation that when opioid dependent mice were allowed to go into a withdrawal state and due to the abstinence of more morphine, that one of the findings was a depletion of epinephrine from the adrenal gland. This raises the question of a possible dysfunction in at least the Sympathetic Branch of the Autonomic Nervous System. And again, this was 1963. What dysfunction led to the depletion of epinephrine in the adrenal glands and during opioid withdrawal? That was the next question.

Our next study for review is by Akera and Brody and from 1968 ***(Figure 7)***. Once again, mice physically dependent upon the opioids were studied. Among the findings by Akera and Brody was an increased level of particularly epinephrine detected in urine of the mice and during a state of opioid withdrawal due to a withholding of the opioids.

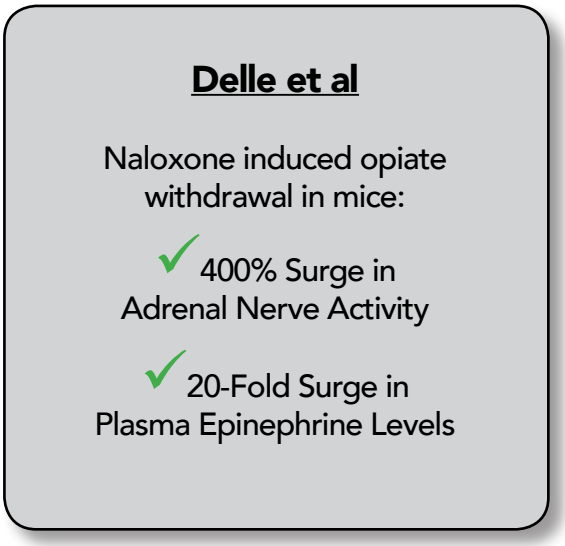
"During withdrawal after chronic (opioid) drug treatment, larger amounts of epinephrine and norepinephrine were excreted, epinephrine being the primary free amine excreted." Akera and Brody (1968)

With these two studies we now have a strong suggestion that in a state of opioid withdrawal, epinephrine is being released from the adrenal glands and in a large concentration. So large in fact, that the epinephrine in the adrenal gland is essentially depleted in a couple of days. This would certainly be suggestive of a dysfunction within the Sympathetic Nervous System. But at this point in time, no direct measurements on the various branches of the Sympathetic Nervous System had been undertaken. For this information, we had to wait until the year 1990. And in the year 1990, we see two excellent studies reported.



(Figure 8)

The first of the two studies from 1990 is the Delle et al study ***(Figure 8)***. Delle (1990) is a complex study with multiple variables. And again, Delle is studying opioid dependent mice and during a state of opioid withdrawal. In the Delle study, opioid withdrawal was accomplished not by a period of opioid abstinence but rather by administration of naloxone, a common opioid antagonist. Either method produces a state of opioid withdrawal. And it is important to note in every study exactly how the opioid withdrawal was obtained, either by a period of opioid abstinence or by the administration of naloxone.



(Figure 9)

But we feel, and apparently Delle et al agree, that the single most significant finding came from a direct measurement of the Sympathetic Nerve innervating the adrenal glands along with a direct measurement of plasma epinephrine levels. This type of direct measurement of the activity of the sympathetic nerve to the adrenal gland could only be accomplished surgically. And this is precisely what Delle and team accomplished ***(Figure 9)***. And what they discovered is nothing short of remarkable. Delle and team discovered a 400% increase in Sympathetic Nerve Activity specifically to the branches of the Sympathetic Nervous System innervating the adrenal glands and during the naloxone induced opioid withdrawal. This is truly a remarkable scientific achievement. Corresponding to this 400% surge in Sympathetic Nerve Activity to the adrenal glands was a simultaneous twenty-fold surge in plasma epinephrine levels. Delle (1990) greatly advanced our knowledge of dysfunction within the Autonomic Nervous during opioid withdrawal. But three strong questions remained after the Delle (1990) study:

1. Would the same type of surge in activity in the sympathetic nerve to the adrenal gland be seen in a withdrawal state obtained simply by opioid abstinence? The Delle (1990) study only utilized mice in opioid withdrawal due to the administration of naloxone.

2. Would a similar increase in plasma epinephrine levels be seen in a withdrawal state obtained simply by opioid abstinence? Again, the Delle(1990) study only utilized mice in opioid withdrawal due to the administration of naloxone.

3. What evidence do we have that the rise in plasma epinephrine is from epinephrine actually derived from the adrenal gland?

Role of plasma catecholamines in eliciting cardiovascular changes seen during naloxone-precipitated withdrawal in conscious, unrestrained morphine-dependent rats

A P Chang et al. J Pharmacol Exp Ther. 1990 Sep.

"After removal of adrenal glands from morphine-dependant rats, naxolone injection produced no change in the BP or plasma Epi."

(Figure 10)

And to address these three questions we turn to the second study from 1990, this time by Chang et al (1990) ***(Figure 10)***. Again, like Delle (1990), the Chang (1990) study is complex and multifaceted. But we remain focused on scientific evidence for dysfunction within the Autonomic Nervous System during a state of opioid withdrawal. And we find plenty of scientific evidence within the Chang (1990) study. Let's take each of the three questions separately and one at a time.

1. Would the same type of surge in the activity in the sympathetic nerve to the adrenal gland be seen in a withdrawal state obtained simply by opioid abstinence? Chang (1990) DOES NOT answer this question as Chang did not do surgical measurements of the sympathetic nervous system. So we simply do not have any further knowledge as to this question.

Chang et al

Naloxone induced opiate withdrawal in mice:

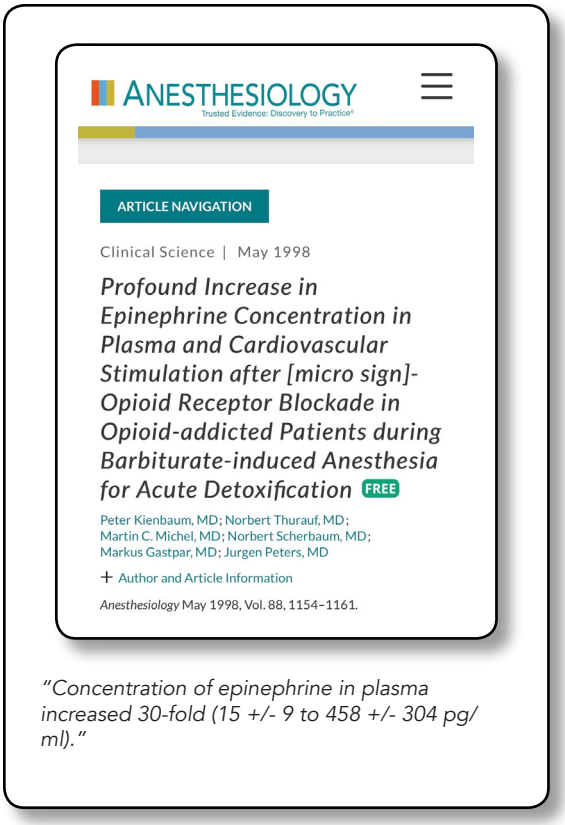
✓ Opioid abstinence withdrawal increased plasma epinephrine levels

✓ Surgical resection of the adrenal glands in mice prevented the surge in plasma epinephrine levels in opioid withdrawal

(Figure 11)

2. Would a similar increase in plasma epinephrine levels be seen in a withdrawal state obtained simply by opioid abstinence? And here Chang (1990) does provide an answer. And this answer is YES ***(Figure 11)***. Chang (1990) found that plasma epinephrine levels increased daily over the three days of measurement (Day 0, 0.183 +/- 0.038 ng/ml), (Day 1 of opioid abstinence withdrawal, 0.665 +/- 0.061 ng/ml), (Day 2 of opioid abstinence withdrawal, 0.730 +/- 0.071 ng/ml), (Day 3 of opioid abstinence withdrawal, 1.00 +/- 0.091 ng/ml). This is strong scientific evidence. And this would be highly consistent with the findings of epinephrine depletion from the adrenal glands due to opioid withdrawal in the Gunne (1963) study. And this would be highly consistent with the findings of epinephrine in the urine due to opioid withdrawal in the Akera and Brody (1968) study.

3. What evidence do we have that the rise in plasma epinephrine is from epinephrine actually derived from the adrenal gland? And once again, Chang et al provide clarity through scientific evidence. Chang et al simply repeated the naloxone induced opioid withdrawal but this time in mice whom had undergone a surgical resection of the adrenal glands. And when the adrenal glands had been surgically removed, there was no surge in plasma epinephrine levels following the administration of naloxone to opioid dependent mice. An answer had been obtained. And this is taken as strong scientific evidence that the measured surge in plasma epinephrine levels during opioid withdrawal is from epinephrine actually derived from the adrenal glands.



(Figure 12)

The next study for our review was reported by Kienbaum et al in 1998 ***(Figure 12)***. And this will be the first study involving human participants. So far, we have focused on studies involving mice as participants. But from the mice we have learned much. We first learned that epinephrine was depleted from the adrenal gland during opioid withdrawal (Gunne (1963) ***(Figure 13)***. We then saw that elevated epinephrine was seen in the urine of mice during opioid withdrawal (Akera and Brody (1968)). Our knowledge was greatly expanded by Delle (1990) who gave us the scientific evidence that opioid withdrawal was associated with a 400% increase in Sympathetic Nerve Activity in the branch of the sympathetic nerve going to the adrenal gland. Furthermore, this markedly increased Sympathetic Nerve Activity was associated with a twenty-fold surge in plasma epinephrine levels and all during a naloxone-induced state of opioid withdrawal. Lastly with the mice studies, we learned from Chang (1990) that the elevated plasma epinephrine levels were present regardless if the state of opioid withdrawal was due to the administration of naloxone or if the state of opioid withdrawal was due to a period of opioid abstinence. Furthermore, surgical removal of the adrenal glands prevented the surge in plasma epinephrine levels. This was taken as strong scientific evidence that it was the surge in the Sympathetic Nerve Activity that was producing the surge in plasma epinephrine levels. And it is with this knowledge base that we now encounter our first study involving human participants.

Kienbaum et al (1998) was looking to determine if it was safe to purposely put a human subject into a naloxone induced state of opioid withdrawal. At the time, 1998, and still today, a small minority of healthcare professionals advocate for a treatment form known as the rapid opioid detoxification. Further discussion of this treatment is beyond the scope of this manuscript. Kienbaum was merely trying to assess the safety of an opioid withdrawal, in this instance one induced by the administration of naloxone. Kienbaum was careful in his final assessment and said merely:

What We HAVE Learned From the Mice Studies

✓ Plasma epinephrine surges in **BOTH** Naloxone-induced withdrawal **AND** opioid abstinence withdrawal (Delle 1990, Chang 1990)

✓ Epinephrine is depleted from the adrenal gland by opioid withdrawal (Gunne 1963)

✓ Epinephrine increases in the urine during opioid withdrawal (Akera, Brody 1968)

✓ Surgical removal of the adrenal glands prevents the surge in epinephrine during opioid withdrawal

(Figure 13)

"Most important, a 30-fold increase in concentration of epinephrine in plasma, a small increase in concentration of norepinephrine in plasma, and profound cardiovascular alterations were observed after mu-opioid receptor blockade despite maintenance of general anesthesia. Because of the attendant cardiovascular stimulation, we suggest that acute detoxification of patients addicted to opioids should be performed by trained anesthesiologists or intensivists."
Kienbaum (1998)

What We HAVE Learned From the Human Studies

✓ Naloxone-induced opioid withdrawal is associated with a "profound" surge in plasma epinephrine

? But what about opioid abstinence withdrawal? Is there also an increase in plasma epinephrine in humans in opioid abstinence withdrawal?

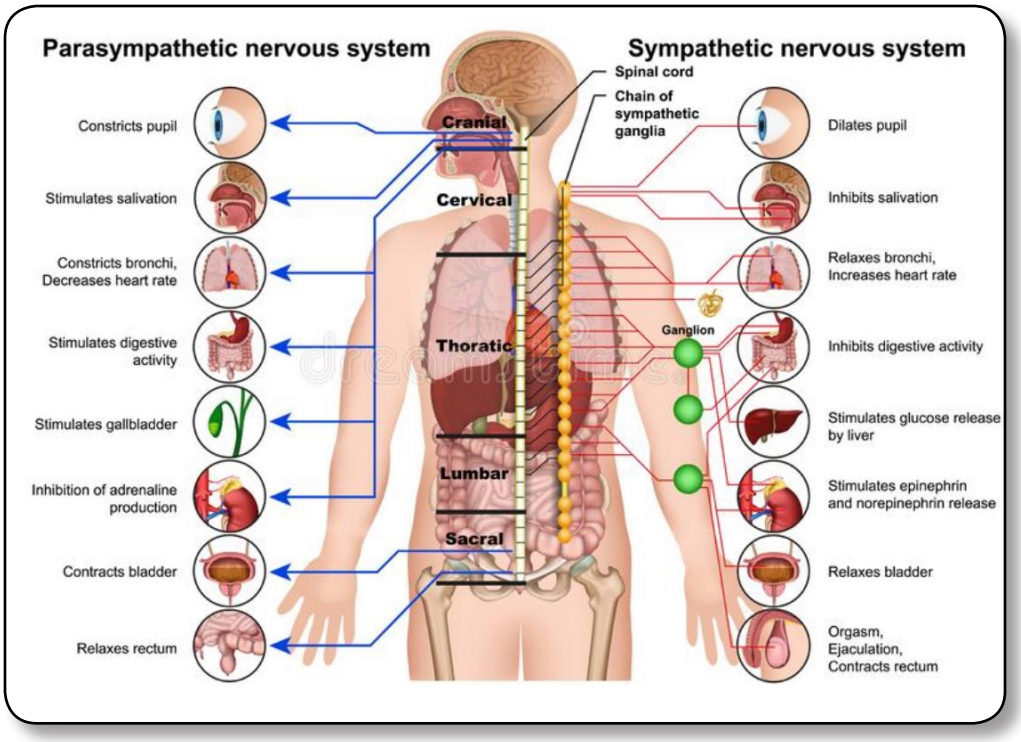
THAT IS THE PURPOSE OF THIS CLINICAL TRIAL

(Figure 14)

As is evident from this quote, Kienbaum found a thirty-fold surge in plasma epinephrine levels during a naloxone induced state of opioid withdrawal. Furthermore, Kienbaum further reported "profound cardiovascular alterations". The emphasis is on the word "profound". It is worth noting, Kienbaum in 1998 was advising naloxone be administered only by "trained anesthesiologists and intensivists". Contrast these words to today when naloxone is carried by all paramedics, many police officers, and some citizens. Granted, this is for use only in lifesaving situations and has saved many lives. But it raises an interesting question.

Before we leave the Kienbaum (1998) study, it is worth the time to note an additional finding made by Kienbaum and team:

"The clinical signs of [micro sign]-opioid receptor blockade were observed in all patients: marked gastrointestinal secretion with 500–1,000 ml of fluids draining from the gastric tube and rectal discharges of 200–500 ml during the 180-min observation period." Kienbaum (1998)



(Figure 15)

Why are these findings of "marked gastrointestinal secretion.....and rectal discharges" deemed to be so important? Because these are known to be bodily functions associated with the Parasympathetic Nervous System, the other branch of the Autonomic Nervous System ***(Figure 15)***. We saw direct measurements made surgically upon the mice during opioid withdrawal that revealed a marked dysfunction in the Sympathetic Nerve Activity in the Sympathetic Nerve to the adrenal glands Delle (1990). This dysfunction within the Sympathetic Nervous System resulted in a twenty-fold surge in plasma epinephrine levels. And this was taken to be excellent and direct evidence of dysfunction within the Sympathetic Nervous System, one of the two branches of the Autonomic Nervous System. Why do we say this is direct evidence? Because Delle (1990) surgically measured the activity in various areas of the Sympathetic Nervous System. This was how the 400% surge in activity in the Sympathetic Nerve going to the adrenal glands was measured, by direct measurement. The other branch of the Autonomic Nervous System is the Parasympathetic Nervous System. And with these large amounts of gastric secretions and rectal discharge, we are seeing indirect evidence suggesting a dysfunction also occurring within the Parasympathetic Nervous System. We are not aware of any studies done to date revealing direct measurements of any branches of the Parasympathetic Nervous System. The type of direct measurement that Delle (1990) did upon the Sympathetic Nervous System has simply not been done to date on the Parasympathetic Nervous System.

COWS Clinical Opiate Withdrawal Scale

Resting Pulse Rate: _____beats/minute *Measured after patient is sitting or lying for one minute* 0 Pulse rate 80 or below 1 Pulse rate 81-100 2 Pulse rate 101-120 4 Pulse rate greater than 120	GI Upset: *over last 1/2 hour* 0 No GI symptoms 1 Stomach cramps 2 Nausea or loose stool 3 Vomiting or diarrhea 5 Multiple episodes of diarrhea or vomiting
Sweating: *over past 1/2 hour not accounted for by room temperature or patient activity.* 0 No report of chills or flushing 1 Subjective report of chills or flushing 2 Flushed or observable moistness on face 3 Beads of sweat *on* brow or face 4 Sweat streaming off face	Tremor *observation of outstretched hands* 0 No tremor 1 Tremor can be felt, but not observed 2 Slight tremor observable 4 Gross tremor or muscle twitching
Restlessness *Observation during assessment* 0 Able to sit still 1 Reports difficulty sifting still, but is able to do so 3 Frequent shifting or extraneous movements of legs/arms 5 Unable to sit still for more than a few seconds	Yawning *Observation during assessment* 0 No yawning 1 Yawning once or twice during assessment 2 Yawning three or more times during assessment 4 Yawning several times/minute
Pupil size 0 Pupils pinned or normal size for room light 1 Pupils possibly larger than normal for room light 2 Pupils moderately dilated 5 Pupils so dilated that only the rim of the iris is visible	Anxiety or irritability 0 None 1 Patient reports increasing irritability or anxiousness 2 Patient obviously irritable anxious 4 Patient so irritable or anxious that participation in the assessment is difficult
Bone or Joint aches *If patient was having pain previously, only the additional component attributed to opiates withdrawal is scored* 0 Not present 1 Mild diffuse discomfort 2 Patient reports severe diffuse aching of joints/ muscles 4 Patient is rubbing joints or muscles and is unable to sit still because of discomfort	Gooseflesh skin 0 Skin is smooth 3 Piloerrection of skin can be felt or hairs standing up on arms 5 Prominent piloerrection
Runny nose or tearing *Not accounted for by cold symptoms or allergies* 0 Not present 1 Nasal stuffiness or unusually moist eyes 2 Nose running or tearing 4 Nose constantly running or tears streaming down cheeks	Total Score _____ The total score is the sum of all 11 items Initials of person completing Assessment:_____
Score:	5-12 mild; 13-24 moderate; 25-36 moderately severe; more than 36 = severe withdrawal

(Figure 16)

But even without the direct measurement of dysfunction within the Parasympathetic Nervous System, we do have evidence to further consider. First, we can evaluate the symptoms seen clinically in opioid withdrawal. Whether or not the opioid withdrawal is due to the administration of an opioid receptor antagonist such as naloxone or if the opioid withdrawal is due simply to a period of opioid abstinence, the clinical signs and symptoms are the same. As a guidance, we shall use the Clinical Opiate Withdrawal Scale, commonly referred to as the COWS ***(Figure 16)***. And we shall go down the list and classify each entry into the COWS as either sympathetic, parasympathetic, or other:

Changes in cardiac vagal tone as measured by heart rate variability during naloxone–induced opioid withdrawal

Charles J Levin et al. Drug Alcohol Depend. 2019.

CONCLUSIONS: These preliminary data indicate that a large reduction in cardiac vagal tone occurs during naloxone-induced withdrawal. This finding underscores the need for further research into the role of the parasympathetic nervous system in opioid withdrawal.

(Figure 17)

Pulse rate - sympathetic; sweating - sympathetic; restlessness - sympathetic; pupil size - sympathetic; bone/joint aches - other; runny nose - parasympathetic; tearing - parasympathetic; vomiting - parasympathetic; diarrhea - parasympathetic; tremor - sympathetic; yawning - parasympathetic; anxiety/irritability - sympathetic; goose-flesh skin - sympathetic.

And we can see that other than an abnormality in pain perception, the entire COWS can be seen as nothing more than a dysfunction within both branches of the Autonomic Nervous System, the Sympathetic and Parasympathetic Nervous Systems. Amazingly, and to the best of our knowledge, this is simply the first time the COWS has ever been viewed as merely evidence for dysfunction within the Autonomic Nervous System. This raises the possibility that the opioids, if taken long enough, are producing a dysfunction within a major organ system - the Autonomic Nervous System.

Before we leave our discussion of the Parasympathetic Nervous System, there is one additional study available for our consideration. We turn our attention now to a study by Levin et al (2019) ***(Figure 17)***. Levin worked with opioid dependent human subjects put into a state of opioid withdrawal by the administration of an intramuscular injection of naloxone. Levin was looking to gain some level of understanding of a dysfunction within the Parasympathetic Nervous System during opioid withdrawal. This study utilized a relatively new technique for evaluation of the Autonomic Nervous System - the Heart

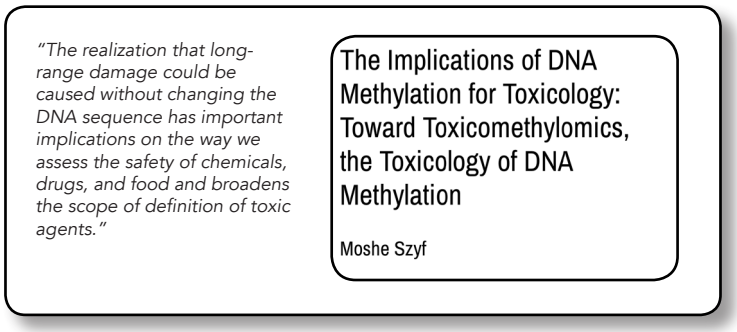
Rate Variability (HRV). Decreased HRV is believed to be a function of both increased sympathetic nerve activity and decreased parasympathetic nerve activity. And we can see here the findings by Levin et al (2019):

<u>CONCLUSIONS:</u> These preliminary data indicate that a large reduction in cardiac vagal tone occurs during naloxone-induced withdrawal. This finding underscores the need for further research into the role of the parasympathetic nervous system in opioid withdrawal.

Clearly Levin and the fellow researchers believed they were seeing an immediate dysfunction within the Parasympathetic Nervous System during opioid withdrawal. But what is it that the opioids were doing that could cause such a major dysfunction within both branches of the Autonomic Nervous System? We turn now to our second area of study - opioid induced methylation within the promoter region of the OPRM1 gene.

OPIOID INDUCED METHYLATION WITHIN THE PROMOTER REGION OF THE OPRM1 GENE

We shall begin this discussion with a quick review of the excellent article by Moshe Szyf and from 2011 ***(Figure 18)***. Szyf 2011 was viewing methylation within the DNA as a form of toxicity to the body:



(Figure 18)

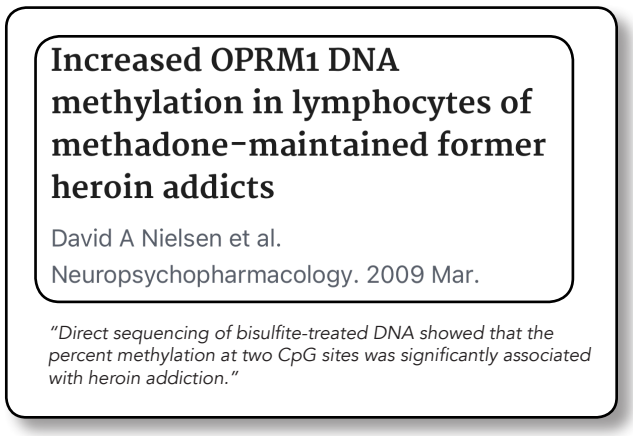
"The realization that long-range damage could be caused without changing the DNA sequence has important implications on the way we assess the safety of chemicals, drugs, and food and broadens the scope of definition of toxic agents."

And we wish to draw the readers attention to the phrase "long-range damage" There is simply no better way to describe the toxicity of methylation than the descriptive phrase "long-range damage". Are the opioids causing the type of long-range damage of which Szyf was warning? The answer is a resounding- yes. This toxicity to the DNA known as methylation has been well studied. We present now, and briefly, the scientific evidence of the opioids and DNA methylation. We have divided the scientific evidence into three broad categories: association, correlation, and causation between the opioids and DNA methylation.

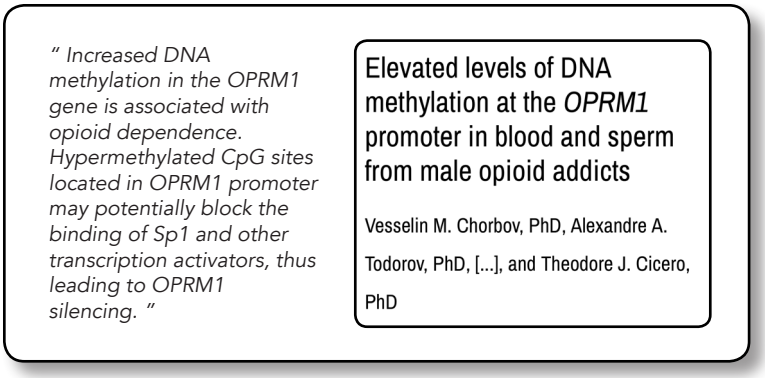
ASSOCIATION BETWEEN THE OPIOIDS & DNA METHYLATION WITHIN THE PROMOTER REGION OF THE OPRM1 GENE

We turn first to two separate studies that we feel are reflective of an association between the opioids and DNA methylation within the promoter region of the OPRM1 gene. The first study is by Nielsen et al and from 2008. Nielsen (2008) studied participants formerly using heroin, now stabilized on Methadone ***(Figure 19)***. Nielsen compared the DNA of these participants to a set of controls who had no dependency upon any of the opioids. And what Nielsen found was astonishing:

"Both the -18 and the +84 CpG sites are located in potential Sp1 transcription factor-binding sites.



(Figure 19)



(Figure 20)

Methylation of these CpG sites may lead to reduced OPRM1 expression in the lymphocytes of these former heroin addicts." Nielsen (2008)

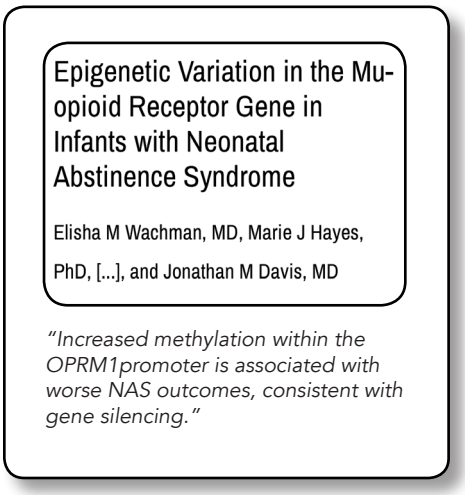
Not only was methylation present within the promoter region of the OPRM1 gene, but methylation was present within the actual SP1 binding sites. This type of gene silencing could be of a high significance. The OPRM1 gene encodes for the (mu) opioid receptor. Methylation within the promoter region, and more specifically, within the SP1 binding sites could be a disaster for the individual. (Note, detailed discussion of methylation, promoter region, gene silencing, and SP1 binding sites is beyond the scope of this manuscript.)

Could this most important of scientific findings be replicated? The answer again is- yes. These findings were replicated and by Chorbov et al and in 2011 ***(Figure 20)***. Chorbov also studied opioid dependent volunteers from the local Methadone Clinic and compared these opioid dependent participants to a set of controls who were not opioid dependent. By so doing, Chorbov replicated the findings of Nielsen:

<u>CONCLUSIONS:</u> "Increased DNA methylation in the OPRM1 gene is associated with opioid dependence. Hypermethylated CpG sites located in OPRM1 promoter may potentially block the binding of Sp1 and other transcription activators, thus leading to OPRM1 silencing."

We now have seen replicating scientific evidence for an association between the opioids and DNA methylation within the promoter region of the OPRM1 gene. We next turn our attention to the concept of a correlation between the opioids and DNA methylation within the promoter region of the OPRM1 gene. In other words, we know that the methylation is present in those individuals dependent upon the opioids. But is there any scientific evidence that this methylation is actually doing anything to the body? And for this concept of correlation between the opioids and DNA methylation within the promoter region of the OPRM1 gene, we turn now to work done by Dr. Elisa Wachman and at the Boston Medical Center.

<u>CORRELATION BETWEEN THE OPIOIDS AND DNA METHYLATION WITHIN THE PROMOTER REGION OF THE OPRM1 GENE</u>



(Figure 21)

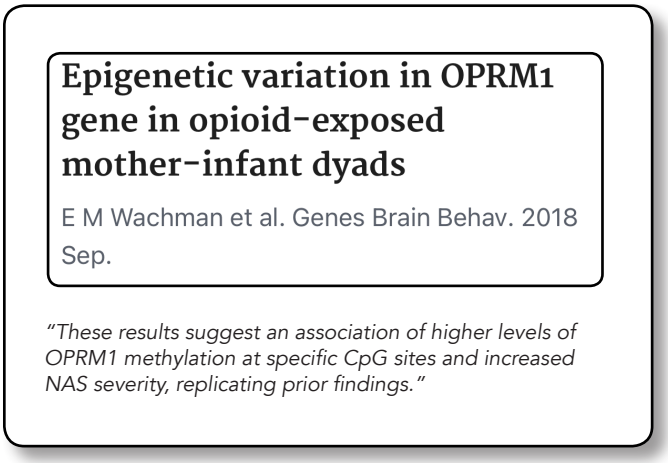
The first study by Dr. Wachman et al is from 2014 ***(Figure 21)***. Dr. Wachman is a pediatrician. Specifically, Dr. Wachman studied the infants exposed to opioids during their gestation and were therefore at risk for opioid withdrawal following delivery. Opioid withdrawal in the newborn is known as Neonatal Abstinence Syndrome (NAS). In her own words:

"We correlated DNA methylation levels in the mu-opioid receptor (OPRM1) promoter in opioid-exposed infants and correlate them with NAS outcomes."

This is to say, Wachman (2014) was looking for a correlation between the level of methylation measured in the infant and the severity of the symptoms experienced by the infant. And again, it is to be

emphasized, this methylation is found within the promoter region of the OPRM1 gene. And again, further emphasize is placed by Dr. Wachman on the possible role of this methylation to the SP1 binding sites within the promoter region of the OPRM1 gene ***(Figure 21)***. In the conclusion, Dr, Wachman states:

<u>CONCLUSIONS:</u> "Increased methylation within the OPRM1 promoter is associated with worse NAS outcomes, consistent with gene silencing."



(Figure 22)

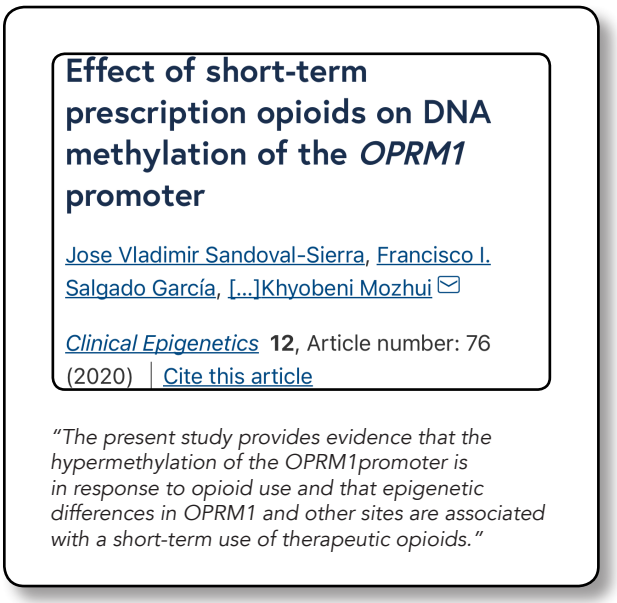
It is worth the time to read carefully the words within this conclusion by Wachman (2014). What these words are implying is the basic scientific concept of Cause and Effect. In essence, the methylation is the cause of the symptoms known widely as opioid withdrawal. We feel that the implications of these findings have not been fully realized to date by either the medical community at large, governmental leaders, or the general population.

Dr. Wachman must have felt that these findings were of real scientific importance. A second study was undertaken and completed in 2018. This second study, Wachman (2018) was quite similar to the first ***(Figure 22)***. The main difference in the second study is that Dr. Wachman chose to correlate not only the infants level of methylation within the promoter region of the OPRM1 gene to the severity of the withdrawal symptoms but also to correlate the mothers level of methylation within the promoter region of the OPRM1 gene and the severity of the withdrawal symptoms experienced by the infant. And here are the findings:

"This study shows associations between maternal and infant methylation levels in the OPRM1 promoter region and differences in NAS severity. Higher levels of methylation were observed at several CpG sites in infants who required pharmacologic treatment and correlated with infant LOS. These results obtained in an independent cohort confirm our prior findings in an independent cohort and, for the first time, show an association with maternal methylation levels and NAS severity."

The second Wachman (2018) study was successful in both confirming the prior correlation between infant methylation and severity of withdrawal and, for the first time, showing an association between maternal methylation and the severity of the withdrawal symptoms in the infant. We now have strong scientific evidence for the association between the opioids and DNA methylation (Nielsen (2008), Chorbov (2011)). We now have strong scientific evidence for the correlation between the opioids and DNA methylation (Wachman (2014) Wachman (2018)). The next step from the scientific perspective is evidence that it is the opioids themselves that are causing the DNA methylation. In other words, we need the scientific proof that when an individual takes the opioid, the methylation occurs as a result of the opioid exposure. This is known scientifically as causation. And for scientific evidence of causation, we turn to a study from 2020.

CAUSATION BETWEEN THE OPIOIDS & DNA METHYLATION WITHIN THE PROMOTER REGION OF THE OPRM1 GENE



(Figure 23)

We have now seen scientific evidence for both an association and a correlation between the opioids and DNA methylation within the promoter region of the OPRM1 gene. The next question is whether or not it is the opioids themselves that are causing the DNA methylation. This would be known as causation. Do we have any scientific evidence for causation? This would require the evaluation of an opioid-naive individual who then takes the opioid and with DNA testing for methylation before, during, and after treatment with the opioids. And fortunately, we have exactly this study and done recently by Dr. Jose Vladimir Sandoval-Sierra et al and in 2020 ***(Figure 23)***. Sandoval-Sierra (2020) studied opioid-naive individuals undergoing dental surgery. Three DNA samples were obtained: before, during, and after standard treatment with the opioids. And the findings are highly significant. The opioids appear to be highly toxic to the DNA of humans and with measurable levels of methylation occurring with just a few doses. Apparently, none are immune to this methylation.

CONCLUSIONS: "The present study provides evidence that the hypermethylation of the OPRM1 promoter is in response to opioid use, and that epigenetic differences in OPRM1 and other sites are associated with short-term use of therapeutic opioids."

Again, we ask the reader to simply stop and think of the implications of these words. Look again at the quote "the hypermethylation of the OPRM1 promoter is in response to opioid use". We are now at a point wherein we have excellent scientific evidence for the triad of association, correlation, and causation between the opioids and DNA methylation within the promoter region of the OPRM1 gene.