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Thesis

CLASSIFYING THE MANNER OF DEATH IN DRUG / ETHANOL OVERDOSE
IN EQUIVOCAL CASES: A SUGGESTED FUTURE TOOL FOR MEDICAL
EXAMINERS USING NEUROANATOMICAL MARKERS

by

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CLASSIFYING THE MANNER OF DEATH IN DRUG / ETHANOL OVERDOSE IN EQUIVOCAль Cases: A SUGGESTED FUTURE TOOL FOR MEDICAL EXAMINERS USING NEUROANATOMICAL MARKERS

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ABSTRACT

The purpose of the present thesis was to propose a guideline to differentiate between an accidental or suicide manner of death when dealing with a drug/ethanol overdose in which all available medical and investigational evidence, including a psychological autopsy, is inconclusive, thereby resulting in an undetermined manner of death. An in-depth literature review was conducted in the field of neuroscience, psychiatry, and pharmacology to discover neuroanatomical markers indicative of suicidal behavior in the context of two major risk factors of suicide, stress and depression, and two hypotheses behind the cause of suicidal behavior, impulsive aggression and neuronal plasticity. The neuroanatomical markers of suicidal behavior, as indicated by the experimental evidence of various studies in suicide subjects, included serotonergic dysfunction, hypothalamic-pituitary-adrenal axis hyperactivity, brain-derived neurotrophic factor deficiency, and the associated anatomical changes in the
brain. Upon consideration of the forensic applicability of analyzing these neuroanatomical markers indicative of suicidal behavior, a guideline was generated to differentiate between an accidental and suicide manner of death by showing suicide subjects had significantly decreased messenger ribonucleic acid and protein levels of presynaptic serotonin receptors along with significantly increased messenger ribonucleic acid and protein levels of postsynaptic serotonin receptors in the prefrontal cortex, significantly decreased serum brain-derived neurotrophic factor levels, and significantly decreased messenger ribonucleic acid and protein levels of brain-derived neurotrophic factor and tyrosine kinase B receptors in the prefrontal cortex and hippocampus when compared to the levels of both depressed non-suicidal individuals and healthy controls. Given the significant difference observed between suicide subjects and controls, these differences in neuroanatomical markers may play an important role in the pathophysiology of suicidal behavior and have the potential to be used in establishing the intention of an individual in an overdose death to distinguish between an accidental or suicidal manner of death.
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LIST OF ABBREVIATIONS

5-HIAA: 5-Hydroxyindoleacetic acid, the main metabolite of serotonin

5-HTT: Serotonin transporter

5-HT2A: Serotonin receptor

ACTH: adrenocorticotropin hormone

β: beta

BDNF: Brain-derived neurotrophic factor

°C: Celcius

cm: centimeter

CSF: cerebrospinal fluid

CRF: corticotrophin-releasing factor

HPA: hypothalamic-pituitary-adrenal

mRNA: messenger ribonucleic acid

PCR: polymerase chain reaction

RNA: Ribonucleic acid

SSRI: Selective serotonin reuptake inhibitor

trk B: Tyrosine kinase B
1. INTRODUCTION

Death is one of the inevitable events that have always been a target for explanation and understanding. Theorized by intellectuals, researched by scientists, and respected by the religious, death has been interpreted in a variety of ways throughout the ages by personal, scientific, and religious beliefs. For medical examiners, it is their duty and profession to understand the physical mechanics behind an individual’s death and explain why the fatal event occurred through the combination of an autopsy, history review, and death scene investigation. Although medical examiners may not always personally conduct the death scene investigation or interview the next of kin for the deceased’s social, medical, and psychiatric history, which are tasks typically left to medicolegal investigators, medical examiners are solely responsible for performing the medico-legal autopsy.

The medico-legal autopsy generally begins with the external examination of the body. After the collection of trace evidence, removal of clothing, clean-up, and photographic documentation, the physical features of the body are recorded and examined for the presence of wounds. The autopsy proceeds with the internal examination of the body where viscera, cavities, and bones are inspected for trauma, deformity, or lesions. Additional toxicological and histological examination may also be performed but at the discretion of the medical examiner [1]. Regardless of any possible variations in the steps taken,
the reason behind the autopsies remains the same; to determine the cause and manner of death.

The cause of death is a general term used to describe the lethal series of events that led up to an individual’s demise. In typical medico-legal practice, the cause of death describes how an individual died and is differentiated into two separate identities: the proximate cause of death and the immediate cause of death [2]. The proximate cause of death refers to the original injury or disease that is responsible for initiating the lethal chain reaction. There can only be one injury or disease responsible for the proximate cause of death. In contrast, the immediate cause of death refers to the medical complications resulting from the proximate cause of death. For example, if an individual was stabbed in a way that was not directly lethal but develops into a fatal case of sepsis and peritonitis days later, the proximate cause of death would be the stab wound and the immediate causes of death would be both sepsis and peritonitis. Compared to the immediate cause of death, the proximate cause of death can be more challenging to establish, especially if separated by an extended period of time, but can be used in the determination of the manner of death.

The manner of death refers to a classification scheme used to differentiate why an individual died. In contrast to the cause of death, which is strongly supported by empirical medical data from autopsies, the manner of death relies more on the combination of death scene findings and third party information such as witness testimonials, police reports, and family interviews. Although there are
no globally accepted standards to how a manner of death is classified [8], five broad categories are recognized to describe the circumstances behind a death: natural, accident, suicide, homicide, and undetermined.

A natural manner of death generally encompasses the deaths caused solely by disease. The term disease may include adverse effects due to chronic abuse to the body from factors such the environment, drugs, genetics, and aging. As a result of the broad definition, the leading majority of deaths annually are comprised of natural deaths. Sometimes, the assignment of a natural manner of death may be complicated, due to sharing similar scenarios as accidental deaths, but are usually differentiated by a measure of time between symptoms and the fatal event. For example, in a fatal case of acute alcohol intoxication, a chronic alcoholic would be assigned a natural manner of death while a teenager who experimented with alcohol for the first time would be assigned an accidental manner of death [2]. The only difference between the two scenarios is that the chronic alcoholic already had adverse medical symptoms, such as liver cirrhosis, developed over a period of time from alcoholism and exacerbated to the point of being lethal, while the teenager did not.

A report of accidental manner of death is less prevalent than natural manner of death and includes all deaths caused by a fatal injury that was not expected or intended. However, the specific requirement for a death to be classified as an accident is proof that the death was not caused intentionally, either by the deceased or by another person [2]. The majority of accidental
manner of deaths can be contributed to impairment, ignorance, or human error with the two leading causes of death, as categorized by the Centers for Disease Control and Prevention mortality database, being motor vehicle traffic and drug intoxications.

A suicide manner of death is more refined in definition and includes all deaths where the decedent was held accountable for the fatal injury. In contrast to accidental manner of death, in order to be classified as a suicide, the fatal injury must be self-inflicted and shown to have been performed intentionally [2]. A reasonable amount of medical certainty must accompany the determination otherwise, due to the difficult nature of suicide and the stigma it bears, the classification of suicide will sometimes be controversial and amended. Therefore, the determination of a suicide manner of death is usually preceded by a psychological autopsy. In a psychological autopsy, medical examiners consider the social and medical details of the deceased’s life for factors that have been shown to be linked to suicide epidemiology. Social details encompass anything within the individual’s social context such as personal difficulties, working conditions, and the level of social interaction. Medical details encompass anything related to the individual’s physical and mental history such as physical ailments, mental disorders, accounts of intentional self-harm, and communication records between health professionals or help line services. The purpose behind the collection of social and medical details is to gather explicit and/or implicit evidence of the decedent’s intention or desire to kill oneself while being
cognizant of the consequences of their actions. The evidence gathered from suicide investigations are typically used to answer the questions if there were the presence of stressful events or mental disorders, previous experiences with suicide, efforts undertaken by the decedent to prevent rescue, and the inappropriate preparation and expression of farewell and impending death in the context of the decedent’s life. The amount of evidence sufficient enough to determine a suicide manner of death is ultimately up to the discretion of the medical examiner [4].

An undetermined manner of death includes deaths which could not be classified under the four other manners of death. The determination of undetermined is used when a death cannot be differentiated between any of the other four manners of death with more than 50% certainty, despite consideration of all available evidence [3]. The classification of undetermined is mainly used when information is insufficient or when the cause of death cannot be determined due to uninformative circumstances such as skeletonized remains. As a result, undetermined manner of death may leave issues unresolved.

Given the professional responsibility of medical examiners and the social, financial, legal, and religious influence a determination of death may have on the lives of the decedent’s next of kin, it is important, from a moral and ethical perspective, that medical examiners base their findings on all the available facts known and their diagnosis is accurate and indisputable, regardless of the consequences. However, the information collected to help establish the cause
and manner of death may not always be useful. As mentioned earlier, during a psychological autopsy, the deceased’s social, medical, and psychiatric histories are collected from next of kin, alongside witness testimonials, and taken into consideration when assigning the manner of death. For some families, the outcome of the diagnosis may affect them negatively for social, financial, religious, or legal reasons, especially with suicide determinations, therefore cooperation during interviews and investigations may not always be expected. As a result, medical examiners may end up with unsubstantiated or even false information. Therefore, until new empirical methods of collecting evidence are discovered, medical examiners may continue to have difficulty in assigning a manner of death, other than undetermined, in cases that can be diagnosed by more than one manner of death based on circumstantial evidence. Although the assignment of undetermined manner of death is the appropriate result when there is insufficient evidence, the current knowledge of differentiating between manners of death can be expanded and improved upon.

The thesis will focus on a very specific example of this scenario, in particular drug/ethanol overdoses, in which all available information is insufficient, even after a psychological autopsy, to distinguish between a suicide and accidental manner of death, therefore labeled as an undetermined manner of death. The occurrence of the proposed scenario of drug/ethanol overdose in conjunction with insufficient evidence may seem limited in scope, a review of the underlying causes of death in the U.S. would suggest otherwise. Over the time
period of 1999-2009, the number of undetermined deaths reported annually in the United States had risen at a steady pace with overdose by drugs and ethanol consistently comprising the majority of the causes of death. Since overdose fatalities by homicide are generally rare and standard toxicological screening, autopsy, or testimony from next of kin can usually exclude a natural manner of death, it would be justifiable to assume the majority of overdose fatalities by drugs and ethanol were assigned an undetermined manner of death because of the difficulty in differentiating between a suicide or accidental manner of death. This assumption has been supported by several studies which have demonstrated an underreporting of suicide and accident as manners of death in overdose deaths while an increase in the assignment of undetermined. In one particular study of undetermined overdose deaths in Utah, the authors abstracted all suicides and one half of all accidental and undetermined overdose deaths for data and using bivariate analyses of the differences in contributing factors between suicides and accidental overdose deaths such as a history of depression and suicidal behavior, a classification tree was created to reclassify undetermined overdose deaths. Using this approach, the authors demonstrated that up to 30% of suicides and approximately 61% of accidental deaths were underreported [6, 7, 15-19].

The differentiation between a suicide and accidental manner of death is typically determined by establishing the intention of the decedent prior to death. Ante-mortem toxicology levels of most drugs and ethanol are estimated in
correlation to measured post-mortem levels which may be subjected to the effects of various post-mortem processes [8-11]. For example, ethanol, which is one of the most abused drugs in the world, may have post-mortem levels that are increased due to microbial fermentation or levels that are decreased due to redistribution [12]. Despite variations in post-mortem levels, medical examiners usually know how to interpret post-mortem drug and ethanol levels and assign a manner of death. However, in conjunction with toxicological testing, as mentioned earlier when defining the manners of death, intention at a legally defined level must also be shown before a death can be labeled as suicide. This proves especially difficult when direct indications of intent are unavailable; suicides are usually solitary acts with no witnesses and suicide notes are only found in approximately 1 out of every 3 suicides [13, 14].

Without explicit or circumstantial evidence of intention, medical examiners may be unable to decide if there is enough compelling information, even after a psychological autopsy, to classify a fatality by drug/ethanol overdose as an accident or suicide; currently, no recognized method exists to differentiate between these two manners of death in this scenario. The thesis will attempt to resolve this difficulty in classification by assessing the biological mechanisms mediating suicidal behavior and highlighting the anatomical differences observed between suicidal and non-suicidal behavior to discriminate between suicide and accidental manner of death.
2. AIM AND OBJECTIVES

The aim of this study was to suggest methods which may be able to increase the accuracy of a medical examiner’s diagnosis with the goal of developing a new approach to the determination process for manner of death in drug/ethanol overdoses where information is insufficient despite complete medical and scene investigation. Three objectives were considered in order to accomplish this goal. The first objective was to identify potential hypotheses to explain the pathophysiology of suicide. The second objective was to present support for these hypotheses through direct and indirect evidence obtained from a thorough literature review. The direct evidence consisted of induced anatomical and neurochemical changes of the brain derived from controlled animal or human studies that simulated predisposing factors of suicidal behavior. The indirect evidence, on the other hand, consisted of circumstantial patterns of differences between suicide subjects and controls from in vivo and post-mortem studies. The third and final objective was to compare these findings and incorporate the validity and forensic applicability of each hypothesis into a method of analysis that could be further researched by the medical community for potential practice.
3. METHODS

3.1. Evaluation of Evidence

To approach the process of formulating a test to prove intention, a literature review was performed to establish a commonality between cases of suicides. The most common association observed from publications was a prevalence of mental illness. Several studies showed a consistently high proportion of suicides, ranging from 68 to 100 percent, were diagnosed with one or more forms of mental illness before the suicide was committed [17, 20, 21, 22, 23]. Since mental illness can be linked to defects in anatomy, it was conjectured that a possible connection could exist between defects in the anatomy of the brain and the intention to commit suicide. Clinical brain function studies were evaluated for potential anatomical differences that can be observed in postmortem scenarios. In addition, past studies have shown a connection between chemical levels in the brain and linked behavior; for example, testosterone, which is a chemical molecule that has its corresponding receptor located in the brain, has been shown to increase aggressive behaviors [24]. Thus, with this example of chemically-mediated behavior in mind, an in-depth literature search was undertaken in established journals within the field of neuroscience, psychiatry, and pharmacology to find potential chemical signaling molecules that may serve as a pathological marker for suicide. In order for a case study to be considered as a supporting item of evidence, the article must
have demonstrated a level of evidence of 3 or lower. Research with level 1 evidence consisted of randomized controlled trials, research with level 2 evidence consisted of non-randomized controlled trials, and research with level 3 evidence consisted of observational studies with controls. Any article that did not satisfy this requirement was excluded from this study.

4. RESULTS / LITERATURE REVIEW

Upon performing the initial literature review, two popular hypotheses were adopted as potential candidates to explain the pathophysiology of suicide. The first hypothesis was characterized by abnormal levels of monoaminergic neurotransmitters and proposed that suicide was the result of the adverse combination between an impulsive-aggressive personality and chronic stress/depression. In comparison, the second hypothesis was characterized by the reduced expression of neurotrophic factor, in particular, brain-derived neurotrophic factor (BDNF), and proposed that suicide occurs when the affected individual’s brain could no longer adaptively respond to stimuli due to a state of reduced neuronal plasticity. Neuronal plasticity refers to the capacity of the brain to change in response to stimuli through the generation and reorganization of neurons. The following review will discuss the supporting literature that demonstrated direct and indirect evidence of the validity and forensic applicability
of each hypothesis in the context of two major risk factors associated with suicide: stress and depression.

4.1. Suicide Risk Factors

Stress

In a biological sense, stress can be defined as the body's physiological and psychological response to a stressor, typically an environmental change or stimuli. The responses are generally mediated by a variety of hormones but two classes of hormones have been recognized as the major contributors. The first class of hormones are the catecholamines with emphasis being placed on epinephrine and norepinephrine. Epinephrine and norepinephrine are two different neurotransmitters that are immediately released from storage in the adrenal medulla and locus ceruleus, respectively, upon the detection of a stressor and are responsible for initiating the series of biochemical changes associated with the fight or flight response.

The second class of hormones is the glucocorticoids which consist of cortisol and corticosterone. After the initial detection of stress, glucocorticoids are released from the adrenal cortex following a hormonal cascade through the hypothalamic-pituitary-adrenal (HPA) axis. In the HPA axis, corticotrophin-releasing factor (CRF) is released from the hypothalamus, resulting in the increase of adrenocorticotropic hormone (ACTH) secretion from the anterior
pituitary, which consecutively induces the release of glucocorticoids from the adrenal cortex [25]. The released glucocorticoids migrate to target tissues throughout the peripheral circulation, bind to intracellular receptors to enter cell nuclei, and either promote or inhibit gene transcription. Compared to the immediate effects of catecholamines, the resulting physiological effects of glucocorticoids are broader, delayed, and more persistent (Figure 1).
Figure 1. Hormonal response to stress by the adrenal cortex and medulla.

Catecholamines (norepinephrine and epinephrine) are released from storage in the adrenal medulla and glucocorticoids are released from the adrenal cortex. Glucocorticoid release is controlled by negative feedback inhibition.

When faced with acute challenges of stress, the extracellular concentration of catecholamines and glucocorticoids become elevated and their combined physiologic effects may help promote the survival of the individual. Upon the termination of the acute stress response, these elevated hormone levels are typically returned to baseline values by their respective system of feedback inhibition [26]. Chronic stress, on the other hand, may exacerbate the physiological changes mediated by catecholamines and glucocorticoids. Normally, under stress free conditions or upon the termination of acute stress, a rise in glucocorticoids stimulates the receptors on the hippocampus, which inhibit CRF release from the hypothalamus through the mechanism of negative feedback, thereby preventing subsequent CRF-stimulated ACTH secretion from the anterior pituitary and ACTH-stimulated glucocorticoid secretion from the adrenal cortex [27, 28]. During chronic stress, on the other hand, the HPA axis becomes hyperactive due to a dysregulation in the negative feedback system of the hippocampus. This dysregulation can be attributed to the combination of the body’s increased energy demands during an extended stress response, the catabolic, or energy producing, nature of glucocorticoids, and the fight of flight
response. A large quantity of glucose is constantly metabolized as an energy source by the large muscles of the body under stressful conditions and glucocorticoids carry out the essential role of inducing changes to meet the metabolic demand by promoting gluconeogenesis. The fight or flight response, on the other hand, inhibits the uptake of glucose in tissues that are not immediately required for survival. When combined together, these glucocorticoid-induced changes elevate the concentration of circulating glucose available for immediate use in the muscles, heart, and brain.

However, this system of energy production may become deleterious as glucose is continuously shunted from vulnerable tissues such as the brain. Since glucose is the only energy source for brain tissue, the depletion of glucose may lead to impaired functionality or even neural degeneration. Several studies have shown that prolonged stress can inhibit glucose transport and metabolism in the hippocampus, which in turn will eventually lead to the downregulation of glucocorticoid receptors in the hippocampus that facilitate the negative feedback response of the HPA axis [29-37]. With this glucocorticoid-induced impairment of the hippocampus, the HPA axis will continue to secrete for the duration of the stress response despite abnormally high levels of circulating glucocorticoids. Although an elevated level of glucocorticoids may enhance survival during acute challenges of stress by mobilizing glucose, a prolonged exposure to glucocorticoids may elicit the degeneration of areas in the brain responsible for averting suicidal behavior.
**Depression**

Depression can generally be defined as a mental state of altered mood characterized by anhedonia, anxiety, fatigue, and self-directed animosity. Depression poses a significant risk factor for suicidal behavior and exhibits physiological adaptations similar to those seen during a chronic stress response. It has been proposed that depression is a byproduct of chronic stress and the similarity in adaptive responses can be attributed to the use of the same effectors in the HPA axis and locus ceruleus, even though the means in which the changes are induced have been indicated to be different [38]. For example, during stress situations, circulating glucocorticoids bind to receptors on the locus ceruleus and increase the activity of tyrosine hydroxylase, an enzyme responsible for the biosynthesis of catecholamines [39]. In acute challenges of stress, this increase in tyrosine hydroxylase activity elevates catecholamine secretion to promote survival; however, in chronic stress, the constant increased activity of tyrosine hydroxylase eventually leads to the depletion of available norepinephrine [40]. In depression, on the other hand, a similar deficiency in norepinephrine secretion is observed and is postulated to be connected to biochemical lesions of the norepinephrine system. These abnormalities, whether genetic, stress-induced, or a combination of both, are broad and include possibilities such as fewer noradrenergic neurons in the locus ceruleus, altered α and β adrenergic cortical receptor binding, and inadequate stimulation of tyrosine hydroxylase activity by glucocorticoids [38, 41-44].
### 4.2. Hypotheses of Suicide Pathophysiology

**Impulsive Aggression and Suicidal Behavior**

The connection between suicidal behavior and personality traits has become an increasingly researched subject over the past few decades. Personality traits, which are molded throughout childhood and stabilized during adulthood, provide a glimpse into how an individual perceives, adapts, and reacts to their environment [45, 46]. Since the act of suicide is reflective of the inability to cope with life situations, such as stress and depression, the difficulty for some individuals to adapt suggests that certain personality traits are predisposed to suicidal behavior. A review of clinical and population research has shown impulsive and aggressive personality traits in suicidal behavior [47-59]. Impulsive and aggressive personality traits, which were characterized by aggressive behavior, impulsivity, and Cluster B borderline personality disorder, were observed in a significant proportion, ranging from 25% to 60%, of subjects with suicide ideation, attempts, and completions (Table 1).

Table 1. Impulsivity and Aggression in Suicide Ideation, Attempters, and Completers According to Individual Studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Total Subjects / Controls</th>
<th>Suicide Ideation</th>
<th>Suicide Attempters</th>
<th>Suicide Completers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Sample Size</td>
<td>Number of Subjects</td>
<td>Aggressive Behavior</td>
<td>Impulsive Aggressive Behavior</td>
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<tr>
<td>-------------------------------</td>
<td>------------------------------</td>
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<tr>
<td>Sarchiapone M, et al.</td>
<td>903 male prisoners</td>
<td>395 (31.9%)</td>
<td>131 (42%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Dumais A, et al.</td>
<td>178 depressed males</td>
<td>N/A</td>
<td>N/A</td>
<td>104 (41.5%)</td>
</tr>
<tr>
<td>Mann JJ, et al.</td>
<td>347 psychiatric patients</td>
<td>N/A</td>
<td>107 (60%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Carli V, et al.</td>
<td>1265 male prisoners</td>
<td>536 (28.5%)</td>
<td>164 (26.8%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Oquendo MA, et al.</td>
<td>308 mood disorder patients</td>
<td>N/A</td>
<td>159 (56.6%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Beautrais AL, et al.</td>
<td>128 suicide attempters / 153</td>
<td>N/A</td>
<td>128 (39.8%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Zouk H, et al.</td>
<td>164 subjects</td>
<td>N/A</td>
<td>N/A</td>
<td>100 (25%)</td>
</tr>
</tbody>
</table>

In addition, the subjects with suicide ideation, attempts, and completions, when compared to controls, generally had a higher score for aggression and impulsivity when measured by the Brown-Goodwin Assessment for Lifetime History of Aggression (BGLHA), Barratt Impulsiveness Scale (BIS), and the Buss-Durkee Hostility Inventory (Table 2). The consistent difference in the level of aggression and impulsivity observed in Table 2 between suicide ideators,
attempters, completers, and controls provide support for a connection between suicidal behavior and personality traits. Furthermore, twin studies have shown the heritability of impulsive aggression through the studies of 800 monozygotic and dizygotic twin pairs raised together or apart, thereby suggesting the possibility of a genetic component for the susceptibility of suicidal behavior in individuals with impulsive-aggressive traits [60, 61]. Since genes are responsible for the particular phenotype of an individual and impulsive aggression has been associated with a high proportion of suicidal behavior, the observation of distinctive neuroanatomical abnormalities in individuals with impulsive-aggressive traits may help explain the pathophysiology of suicide.

Table 2. Mean Aggression and Impulsivity Scores in Suicide Ideation, Attempters, Completers, and Controls According to Individual Studies. BGLHA = Brown-Goodwin Assessment for Lifetime History of Aggression. BIS = Barratt Impulsiveness Scale.

<table>
<thead>
<tr>
<th>Author</th>
<th>Total Subjects / Controls</th>
<th>Suicide Attempters / Completers</th>
<th>Never Attempted / Controls</th>
<th>Suicide Ideation</th>
<th>No Suicidal Ideation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N= 131; BIS Impulsivity Score: 48.50 ± 16.25</td>
<td>N= 772; BIS Impulsivity Score: 46.93 ± 14.74</td>
<td>N= 395; BIS Impulsivity Score: 48.44 ± 15.34</td>
<td>N= 508; BIS Impulsivity Score: 46.14 ± 14.61</td>
</tr>
<tr>
<td>Study Authors</td>
<td>Sample Size</td>
<td>Suicide Completers</td>
<td>Controls</td>
<td>BIS Impulsivity Score</td>
<td>BGLHA Aggression Score</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------</td>
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<td>-----------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Dumais B, et al.</td>
<td>104 suicide completers / 74 controls</td>
<td>N= 69; BIS Impulsivity Score: 67.37</td>
<td>N= 69; BIS Impulsivity Score: 64.34</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>McGirr A, et al.</td>
<td>645 suicide completers / 246 controls</td>
<td>N= 176; BIS Impulsivity Score: 66.52 ± 14.83</td>
<td>N= 176; BIS Impulsivity Score: 67.46 ± 13.63</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Mann JJ, et al.</td>
<td>347 psychiatric patients</td>
<td>N= 106; BIS Impulsivity Score: 54.0 ± 16.1</td>
<td>N= 97; BIS Impulsivity Score: 44.8 ± 15.0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Carli V, et al.</td>
<td>1265 male prisoners</td>
<td>N= 106; BIS Impulsivity Score: 54.0 ± 16.1</td>
<td>N= 97; BIS Impulsivity Score: 44.8 ± 15.0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Oquendo MA, et al.</td>
<td>308 depressed patients</td>
<td>N= 113; BIS Impulsivity Score: 54.7 ± 17.0</td>
<td>N= 107; BIS Impulsivity Score: 46.2 ± 17.7</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Impulsive-aggressive behavior, which is characterized by hostile or injurious behavior in response to provocation, has a well-supported history of being associated with characteristic abnormalities of the frontal lobe, a region of the brain linked to the suppression of emotion [62]. The impaired function of the frontal lobe, due to injury, has been shown to be responsible for modulating impulsive-aggressive behavior and was first recognized in the famous case of Phineas Gage. Initially described as a responsible and socially well-adjusted individual, after surviving a considerable injury in which a tamping rod pierced through his orbital frontal cortex, Phineas Gage became irritable, capricious, and verbally abusive [63-65]. Comparable behavior was observed in returning soldiers of the Vietnam War who suffered penetrating head injuries in the same area [66]. Besides physical injuries, metabolic deficiency of the frontal lobe has also been indicated in impulsive-aggressive behavior. Brain imaging studies of the frontal lobe in violent murderers, psychiatric patients of borderline personality
disorder, and individuals with a history of aggression has exhibited decreased levels of glucose metabolism in comparison to controls [67-70]. For example, a study of local cerebral uptake in glucose using positron emission tomography in 22 murderers and 22 age/gender-matched controls showed lower glucose metabolism in both lateral and medial portions of the prefrontal cortex (Figure 2).

Figure 2. Relative glucose metabolic values for overall left and right medial prefrontal cortex and for overall left and right hemisphere lateral prefrontal cortex in murderer and controls. Data adapted from Raine A et al., 1994.

This deficiency in glucose metabolism can be attributed to an observed reduction in regional cerebral blood flow during an aggressive response. In an imaging study, healthy volunteers, with no history of abnormal behavior,
displayed lower levels of blood flow to the orbital frontal cortex during imagery scenarios that elicited aggressive behavior [71]. Moreover, individuals associated with impulsive-aggressive behavior such as murderers, alcoholics with antisocial personality disorder, and patients with personality disorders showed hypoperfusion of the prefrontal cortex when compared to controls [70, 75, 76].

The reduction in blood flow and glucose metabolism together suggests a functional deactivation of the frontal lobe in impulsive-aggressive personalities and is similar to the significant reduction of local cerebral glucose utilization seen in animal studies of GABA receptor agonist-induced inhibition of cortical neurons [72-74].

In addition to the frontal lobe, the amygdala, a region of the brain implicated in emotional regulation, memory, and attention, has been associated in impulsive-aggressive behavior [77]. With connections to and from the cortical, hypothalamic, and brain stem regions, the amygdala is regarded as a filter for evaluating the significance of a stimuli and mediating the corresponding emotional or behavioral response [78]. It has been suggested that the frontal lobe is in direct control of the amygdala and an inverse correlation exists between the activity of the frontal lobe and the activity of the amygdala. Two functional neuroimaging studies have shown that in healthy normal subjects of 40 women and 14 women and men, respectively, during the suppression of negative emotion the activity of the frontal lobe is significantly increased while the activity of the amygdala is significantly decreased [79, 80]. Therefore, dysfunction of the
frontal lobe may lead to the increased function of the amygdala and the amplification of negative emotional responses. In brain studies using functional magnetic resonance imaging, borderline personality disorder patients, whom commonly exhibit impulsive aggression, were shown to have increased activity in the amygdala and greater startle response, in comparison to healthy subjects, when presented with stimuli designed to elicit negative emotions (Figure 3 and Figure 4) [81-85]. However, when presented with neutral or positive stimuli, the activity of the amygdala and the startle response in borderline personality disorder patients did not differ from healthy controls [85, 86]. This observed pattern of amygdala activity and startle response suggests that impulsive-aggressive individuals are more susceptible to negative stimuli and may explain the difference in resultant behavior between individuals exposed to the same conditions and stimuli.
Figure 3. Functional magnetic resonance imaging (fMRI) of brain regions involved in the voluntary regulation of emotion in response to emotionally aversive stimuli. A. Bilateral amygdala and fusiform gyrus activation to emotionally aversive slides in female patients with borderline personality disorder (left) and in normal volunteers (right). Figure taken from Herpertz SC, et al., 2001. B. Region in which patients with borderline personality disorder showed greater activation towards negative pictures than healthy controls. Figure taken from Koenigsberg HW, et al., 2009.
In conjunction with the decreased activity of the frontal lobe and the increased activity of the amygdala, a difference in levels of neurotransmitters has been observed in the neural circuitry of impulsive aggression. The frontal lobe has been linked to having an inhibitory role on the nucleus accumbens’ dopamine release during amygdala activation and the impaired function of the frontal lobe would attenuate the inhibition, resulting in increased dopamine release [87, 88]. Furthermore, with projections from the amygdala to the locus...
coeruleus, the hyperactivity of the amygdala is speculated to induce a similar increase of activity in the locus coeruleus, thereby elevating levels of released catecholamines, specifically dopamine and norepinephrine [89-92]. Combined together, the resulting high level of dopamine has been implicated in enhancing impulsive-aggressive behavior. From animal studies, increased levels of dopamine, ranging from 130% to 170% above baseline levels, corresponded to impulsive-aggressive behavior in rats that observed or participated in fighting when compared to controls [93-96]. Similarly, in human studies, the cerebrospinal fluid concentration of homovanillic acid (HVA), a metabolite of dopamine, was detected at higher levels in violent offenders when compared to controls [97, 98]. Studies in which dopamine levels were pharmacologically altered provide support for the positive correlation between dopamine levels and impulsive-aggressive behavior. Administration of substances that elevate dopamine concentrations, such as amphetamines, cocaine, alcohol, or dopamine agonists, have been linked to increased levels of aggression, as characterized by the increase in the frequency of fighting [99-104]. On the other hand, the administration of dopamine D2 receptor antagonists, such as antipsychotics agents, have been linked to significantly decreased levels of impulsive aggression in patients with borderline personality disorder as well as schizophrenics, when measured by evaluations such as the Barratt Impulsivity Scale, Buss-Durkee Hostility Inventory, and Aggression Questionnaire [105-108].
Considering that the serotonergic system regulates dopaminergic activity through reciprocal interaction when the frontal lobe is functioning normally, the elevated levels of dopamine observed when the function of the frontal lobe is impaired implies a deficiency of serotonergic function during impulsive-aggressive behavior [109, 110]. Support for this implication was observed in animal studies, in which an increase in dopamine levels was mirrored by a decrease in serotonin levels during and after an aggressive confrontation, and in human studies, in which violent offenders had high levels of dopamine metabolites and low levels of serotonin metabolites [94-98]. Therefore, based on the genetic inheritability of personality traits and the strength of negative correlation between dopamine and serotonin levels, serotonergic dysfunction can be considered a neurochemical indicator of impulsive aggression. The validity of this consideration has been consistently demonstrated through research in animal and human studies. In animal studies, low cerebrospinal fluid (CSF) concentrations of the major serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) has been associated with impulsivity, increased violence during aggressive behavior, and a higher rate of premature death [111-115]. In human studies, summarized in Table 3, lower CSF concentrations of 5-HIAA were found in individuals associated with impulsivity or aggression, such as violent offenders, arsonists, and individuals with personality disorders, when compared to controls. The lowest CSF concentrations of 5-HIAA, however, were observed in individuals associated with both impulsivity and aggression, thereby suggesting serotonergic
dysfunction is most severe during impulsive aggression [116-122]. In addition, the pharmacological manipulation of the serotonin receptors 5-HT_{1A} and 5-HT_{1B} has been shown to play a role in modulating aggression. In mice, the direct or systemic administration of 5-HT_{1A} and 5-HT_{1B} receptor agonists, such as 8-OH-DPAT and CP-94253, into the brain effectively decreased forms of aggressive behavior, such as the frequency of attack bites and pursuits, while the co-administration of 5-HT_{1A} and 5-HT_{1B} receptor antagonists, such as GR-127935 and WAY-100635, blocked these aggression-inhibiting effects [123-132]. Emphasis has been placed on the role of 5-HT_{1B} receptors in mediating aggression and impulse control. In studies comparing mutant mice lacking the 5-HT_{1B} receptor with wild type mice, isolated mutant mice displayed more behavioral disinhibition by attacking intruder mice with higher frequency and intensity than wild type mice [133-135]. Furthermore, the degree of serotonergic activity can be indirectly measured through serum levels of prolactin, a pituitary hormone that is secreted as serotonin concentration increase [136,137]. Studies of central serotonergic function using the administration of fenfluramine hydrochloride, a serotonin releasing agent and uptake inhibitor, showed significantly blunted prolactin response in patients with impulsive aggressive personality disorders when compared to controls, thereby supporting the serotonergic deficiency associated with impulsive aggression [138-140]. Overall, these results provide support for a genetic, although it may also be acquired, predisposition towards suicide because brain imaging studies of
suicide attempters and completers have shown serotonergic abnormalities in the frontal lobe similar to those seen during impulsive aggression [141,142]. Autoradiographic studies of postmortem tissue from suicide completers have suggested the serotonergic dysfunction to be localized in the ventral prefrontal cortex based on having the largest change in numbers of presynaptic and postsynaptic serotonin receptors. In suicides, the numbers of serotonin transporter (5-HTT) sites, which represent the presynaptic serotonin receptors, were shown to be reduced while postsynaptic serotonin receptors, such as 5-HT_{1A} and 5-HT_{2A}, were shown to have increased when compared to controls, thereby indicating a reduction of serotonergic neurotransmission (Figure 5, Figure 6) [143-148]. The increase in postsynaptic serotonin receptors can be viewed as an act of compensatory upregulation in reaction to the decreased activity of the serotonin neurons and is supported by a corresponding increase in protein and mRNA expression [147].
Figure 5. Mean serotonin transporter (5-HTT) binding in Brodmann areas across the prefrontal cortex in suicides and controls. Data adapted from Arango V et al., 1995 and Mann J et al., 2000.
Figure 6. Measures of 5-HT$_{2A}$ receptor mRNA and protein levels in the prefrontal cortex, hippocampus, and nucleus accumbens regions of the brain in normal subjects (n=15) and suicide victims (n=15). Data adapted from Pandey GN et al., 2002.
In addition, since the act of suicide can be considered an act of self-directed aggression, if a correlation existed between impulsive aggression and suicide, then the degree of lethality in a suicide attempt should be proportional to the degree of impulsivity or aggression which can be measured by the level of serotonergic deficiency. Studies of cerebrospinal fluid concentrations of 5-HIAA in suicide attempters have shown lower concentrations in impulsive violent attempters compared to nonimpulsive violent attempters as well as lower concentrations in high lethality suicide attempts compared to low lethality suicide attempts (Table 3) [148-150]. The similarity in decreased serotonin levels observed in suicide and impulsive aggression suggest the possibility that serotonergic dysfunction of the frontal lobe may serve as shared mechanism for the pathogenesis of these two behaviors. Serotonergic dysfunction of the frontal lobe by itself, however, is not enough to explain the pathophysiology of suicide. Therefore, additional insight into neurological mechanisms that predispose individuals into committing suicide may be gained by studying impulsive aggression from the perspective of its relationship with major risk factors of suicide, such as depression and chronic stress.
Table 3. Mean Serotonin Levels in Subjects and Controls According to Individual Studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects : Number</th>
<th>Mean 5-HIAA levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virkkunen M et al., 1987</td>
<td>Arsonists : 20</td>
<td>48.52 ± 18.19 nmol/L</td>
</tr>
<tr>
<td></td>
<td>Violent Offenders : 20</td>
<td>68.05 ± 16.19 nmol/L</td>
</tr>
<tr>
<td></td>
<td>Controls : 10</td>
<td>90.00 ± 29.00 nmol/L</td>
</tr>
<tr>
<td>Virkkunen M et al., 1996</td>
<td>Violent crime or fire setter recidivists : 28</td>
<td>50.8 ± 21.7 pmol/L</td>
</tr>
<tr>
<td></td>
<td>Violent crime or fire setter nonrecidivists : 83</td>
<td>72.5 ± 29.9 pmol/L</td>
</tr>
<tr>
<td>Virkkunen M et al., 1994</td>
<td>Antisocial Personality Disorder : 23</td>
<td>52.5 ± 25 pmol/mL</td>
</tr>
<tr>
<td></td>
<td>Intermittent Explosive Disorder : 20</td>
<td>52.5 ± 25 pmol/mL</td>
</tr>
<tr>
<td></td>
<td>Nonimpulsive Offenders : 15</td>
<td>87.5 ± 17.5 pmol/mL</td>
</tr>
<tr>
<td></td>
<td>Violent offender suicide attempters : 25</td>
<td>58.8 ± 25.2 nmol/L</td>
</tr>
<tr>
<td></td>
<td>Control Subjects : 21</td>
<td>65 ± 20 pmol/mL</td>
</tr>
<tr>
<td>Cremniter D et al., 1999</td>
<td>Impulsive Violent Suicide Attempters : 14</td>
<td>51 ± 41.9 nmol/L</td>
</tr>
<tr>
<td></td>
<td>Nonimpulsive Violent Suicide Attempters : 9</td>
<td>92.4 ± 47.3 nmol/L</td>
</tr>
<tr>
<td>Group</td>
<td>Count</td>
<td>Mean ± SD (nmol/L)</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>-------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Healthy Control Subjects : 23</td>
<td></td>
<td>107.3 ± 57.7</td>
</tr>
<tr>
<td>One time violent crime offender</td>
<td></td>
<td>87.1 ± 23.7</td>
</tr>
<tr>
<td>Multiple time violent crime offender</td>
<td></td>
<td>67.9 ± 12.2</td>
</tr>
<tr>
<td>Impulsive violent offender with suicide attempts : 17</td>
<td></td>
<td>67.4 ± 19.7</td>
</tr>
<tr>
<td>Impulsive violent offender without suicide attempts : 19</td>
<td></td>
<td>91.2 ± 22.0</td>
</tr>
<tr>
<td>Suicide attempters by intoxication or single wrist cut : 22</td>
<td></td>
<td>92.2 ± 27.1</td>
</tr>
<tr>
<td>Depressed suicide attempters : 8</td>
<td></td>
<td>74.5 ± 19.8</td>
</tr>
<tr>
<td>Nondepressed suicide attempters : 22</td>
<td></td>
<td>89.0 ± 28.0</td>
</tr>
<tr>
<td>Suicide by other methods : 8</td>
<td></td>
<td>65.6 ± 11.2</td>
</tr>
<tr>
<td>Healthy controls : 45</td>
<td></td>
<td>103.6 ± 38.3</td>
</tr>
</tbody>
</table>

The comorbidity of impulsive aggressive behavior and depression in suicide has long been recognized by the psychiatric community. Individually, impulsive aggressive behavior and depression are not direct predictors of suicide, however, when comorbid, results in a higher mortality risk for suicide. Case studies of patients with comorbid borderline personality disorder and depression showed the highest degree of lethality and the highest number of
suicide attempts when compared to other subgroups of patients, such as patients with other personality disorders, and controls (Table 4) [151-156]. This synergistic increase in mortality rate, due to comorbidity, may be attributed to the combined utilization of the same neurological mechanisms responsible for the causation of suicide.

Table 4. Suicide attempt characteristics among patients with borderline personality disorder, major depressive episode, or both disorders. Data adapted from Soloff PH et al., 2000.

<table>
<thead>
<tr>
<th>Characteristic of Suicide Attempts</th>
<th>Borderline Personality Disorder (n=25)</th>
<th>Borderline Personality Disorder Plus Major Depressive Episode (n=43)</th>
<th>Major Depressive Episode (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lifetime attempts, covaried for age</td>
<td>2.5 ± 1.8</td>
<td>3.4 ± 2.4</td>
<td>2.3 ± 1.6</td>
</tr>
<tr>
<td>Scores on measures derived from Suicide Intent Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lethal Intent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Most recent attempt</td>
<td>7.9 ± 3.8</td>
<td>8.8 ± 2.9</td>
<td>8.0 ± 3.3</td>
</tr>
<tr>
<td>Lifetime</td>
<td>7.8 ± 4.0</td>
<td>9.5 ± 2.5</td>
<td>8.0 ± 4.1</td>
</tr>
<tr>
<td>Objective planning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Most recent attempt</td>
<td>5.4 ± 3.5</td>
<td>7.6 ± 3.3</td>
<td>7.3 ± 3.5</td>
</tr>
<tr>
<td>Lifetime</td>
<td>5.0 ± 3.4</td>
<td>8.0 ± 2.9</td>
<td>6.4 ± 3.9</td>
</tr>
<tr>
<td>Maximum score on Medical Lethality Scale</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Most recent attempt</td>
<td>2.6 ± 2.3</td>
<td>3.1 ± 1.9</td>
<td>2.3 ± 2.4</td>
</tr>
<tr>
<td>Lifetime</td>
<td>3.5 ± 1.9</td>
<td>3.9 ± 1.6</td>
<td>2.8 ± 2.3</td>
</tr>
</tbody>
</table>

One neuroanatomical abnormality that has been implicated through the use of fenfluramine, in the comorbidity of impulsive aggressive behavior and
depression, is the serotonergic dysfunction of the frontal lobe. Fenfluramine, a serotonin releasing agent and uptake inhibitor, is a chemical agent that stimulates the release of serotonin, which in turn exerts regulatory control over the secretion of pituitary hormones such as prolactin. The plasma levels of these pituitary hormones may serve as an indicator of serotonin responsivity and is widely used to evaluate serotonergic function. Studies of central serotonergic function using fenfluramine challenge had shown significantly lower prolactin response in depressed patients when compared to controls (Table 5) [157-161]. These results are consistent with the blunted prolactin response to fenfluramine challenge in patients with impulsive aggressive personality disorders (Table 5) [138-140]. Further studies of fenfluramine challenge in depressed patients have revealed a potential explanation for the increased mortality rate associated with the comorbidity of impulsive aggressive behavior and depression. Significantly lower prolactin response to fenfluramine was observed in patients with a history of suicide attempts compared to patients without a history of suicide attempts, thereby suggesting another source of serotonergic dysfunction was needed in conjunction with depression to induce suicidal behavior (Table 5) [162, 163].

Table 5. Prolactin Response to Fenfluramine in Subjects and Controls According to Individual Studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects : Number</th>
<th>Prolactin response to fenfluramine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sher L et al., 2003</td>
<td>Unipolar depression : 158</td>
<td>53.3 ± 34.0 (hours x ng/ml)</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td></td>
<td>Bipolar depression : 21</td>
<td>49.8 ± 28.4 (hours x ng/ml)</td>
</tr>
<tr>
<td></td>
<td>Healthy controls : 32</td>
<td>64.6 ± 25.0 (hours x ng/ml)</td>
</tr>
<tr>
<td>Siever LJ et al., 1999</td>
<td>Impulsive-aggressive patients : 6</td>
<td>10.9 ± 11.0</td>
</tr>
<tr>
<td></td>
<td>Normal volunteers : 5</td>
<td>11.0 ± 7.0</td>
</tr>
<tr>
<td>Coccaro EF et al., 1989</td>
<td>Depressed patients with major affective disorder and suicide attempts : 7</td>
<td>5.9 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>Depressed patients with personality disorder and suicide attempts : 7</td>
<td>5.0 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>Depressed patients with major affective disorder and no suicide attempts : 18</td>
<td>10.0 ± 7.0</td>
</tr>
<tr>
<td></td>
<td>Depressed patients with personality disorder and no suicide attempts : 13</td>
<td>9.1 ± 5.5</td>
</tr>
</tbody>
</table>

The serotonergic dysfunction associated with impulsive aggressive behavior holds the potential to account for the serotonergic difference between depression and suicidal behavior. In a fenfluramine challenge study of depressed patients, significantly blunted prolactin response was observed in depressed patients with anger attacks when compared to patients without anger attacks (Figure 7) [164]. The extra serotonergic dysfunction in depressed patients induced by impulsive aggression may represent the observed serotonergic difference, between patients with a history of suicide attempts and patients
without such a history, and justify the increased mortality rate for suicide when depression and impulsive aggressive behavior are comorbid.

Figure 7. Prolactin response to DL-fenfluramine minus placebo in patients with and without anger attacks (AA). Data adapted from Fava M et al., 2000.

In addition to the serotonergic dysfunction of the frontal lobe, other neuroanatomical abnormalities shared by impulsive aggressive behavior and depression include the volumetric reduction of the hippocampus and amygdala. Magnetic resonance imaging studies of patients with borderline personality disorder showed significant reductions in hippocampal and amygdala volumes when compared to controls (Table 6) [165-171]. Similar reductions were observed in patients with major depressive disorder (Table 6) [172-178]. Results
from dexamethasone and corticotrophin-releasing hormone challenge studies suggest the morphological changes are induced by a hyperactive HPA axis. Similar to fenfluramine challenge studies, dexamethasone and corticotrophin-releasing hormone are administered to stimulate the release of ACTH and cortisol, which are used to evaluate HPA activity. In their respective studies, patients with major depression and patients with borderline personality disorder showed significantly enhanced corticotrophin and cortisol response, meaning higher levels of ACTH and cortisol, when compared to controls [179-182]. A study in particular, showed higher responses to dexamethasone and corticotrophin-releasing hormone in depressed patients with a history of attempted suicide compared to depressed patients without such a history (Table 7), thereby suggesting a difference in HPA axis activity that can potentially be accounted for by the comorbidity of depression and impulsive aggressive behavior [182].

Table 6. Mean Hippocampal and Amygdalar Volumes in Subjects and Controls According to Individual Studies. BPD = Borderline Personality Disorder. PTSD = Post Traumatic Stress Disorder.

<table>
<thead>
<tr>
<th>Author</th>
<th>Subjects : Number</th>
<th>Mean Hippocampal Volume</th>
<th>Mean Amygdalar Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schmahl et al., 2003</td>
<td>Patients with BPD : 10</td>
<td>Left side : 2120 ± 416 mm³</td>
<td>Left side : 1665 ± 384 mm³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right side : 1883 ± 286 mm³</td>
<td>Right side : 1661 ± 371 mm³</td>
</tr>
<tr>
<td>Study</td>
<td>Group</td>
<td>Left side</td>
<td>Right side</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------------------</td>
<td>-----------</td>
<td>------------</td>
</tr>
<tr>
<td><strong>Tebartz an Elst L et al., 2003</strong></td>
<td>Controls : 23</td>
<td>Left : 2380 ± 811 mm³</td>
<td>Left : 2167 ± 727 mm³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left : 2244 ± 768 mm³</td>
<td>Left : 2090 ± 723 mm³</td>
</tr>
<tr>
<td></td>
<td>Patients with BPD : 8</td>
<td>Left : 2.82 ± .15 cm³</td>
<td>Left : 1.6 ± .1 cm³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right : 3.07 ± .18 cm³</td>
<td>Right : 1.6 ± .1 cm³</td>
</tr>
<tr>
<td></td>
<td>Healthy Controls : 8</td>
<td>Left : 3.55 ± .29 cm³</td>
<td>Left : 2.1 ± .14 cm³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right : 3.89 ± .37 cm³</td>
<td>Right : 2.15 ± .14 cm³</td>
</tr>
<tr>
<td><strong>Weniger G et al., 2009</strong></td>
<td>Patients with BPD and PTSD : 10</td>
<td>Left : 2.49 ± 0.41 mL</td>
<td>Left : 0.72 ± 0.19 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right : 2.54 ± .46 mL</td>
<td>Right : 0.77 ± .14 mL</td>
</tr>
<tr>
<td></td>
<td>Patients with BPD without PTSD : 14</td>
<td>Left : 2.68 ± 0.37 mL</td>
<td>Left : 0.90 ± .27 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right : 2.84 ± .43 mL</td>
<td>Right : 0.89 ± .24 mL</td>
</tr>
<tr>
<td></td>
<td>Controls : 25</td>
<td>Left : 2.95 ± 0.43 mL</td>
<td>Left : 1.12 ± 0.15 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right : 3.16 ± .35 mL</td>
<td>Right : 1.15 ± .16 mL</td>
</tr>
<tr>
<td><strong>Sala M et al., 2010</strong></td>
<td>Patients with BPD : 15</td>
<td>Left : 1.23 ± 0.23 mL</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right : 1.24 ± .26 mL</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Controls : 15</td>
<td>Left : 1.27 ± 0.22 mL</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right : 1.42 ± .15 mL</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Kronenberg G et al., 2010</strong></td>
<td>Depressed patients : 15</td>
<td>N/A</td>
<td>Left : 1.71 ± 0.34 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N/A</td>
<td>Right : 1.74 ± 0.27 mL</td>
</tr>
<tr>
<td></td>
<td>Controls : 15</td>
<td>N/A</td>
<td>Left : 1.97 ± 0.26 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N/A</td>
<td>Right : 1.97 ± .28 mL</td>
</tr>
<tr>
<td><strong>Sheline YI et al., 1996</strong></td>
<td>Depressed patients : 10</td>
<td>Left : 2159 ± 301 mm³</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right : 2283 ± 324 mm³</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Table 7. Adrenocorticotropic hormone (ACTH) and cortisol response to Dexamethasone (DEX) / corticotrophin releasing hormone (CRH) in suicidal and non-suicidal patients with major depression. BASAL concentrations were defined as the plasma concentration after DEX administration but before infusion of CRH. MAX concentration was defined as the maximum values observed. DELTA values were the difference between MAX and BASAL values. A.U.C is defined as the area under curve. Data adapted from Kunugi H et al., 2004.

<table>
<thead>
<tr>
<th>ACTH response to DEX/CRH (pg/ml)</th>
<th>BASAL</th>
<th>MAX</th>
<th>DELTA</th>
<th>A.U.C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suicidal patient with major depression (n=7)</td>
<td>11.1 ± 5.5</td>
<td>55.1 ± 42.2</td>
<td>44.0 ± 38.0</td>
<td>2657 ± 2027</td>
</tr>
<tr>
<td>Non-suicidal patient with major depression (n=13)</td>
<td>5.3 ± 4.4</td>
<td>23.9 ± 19.1</td>
<td>18.6 ± 16.7</td>
<td>1318 ± 1102</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cortisol response to DEX/CRH (ug/dl)</th>
<th>BASAL</th>
<th>MAX</th>
<th>DELTA</th>
<th>A.U.C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suicidal patient with major depression (n=7)</td>
<td>3.3 ± 2.4</td>
<td>12.7 ± 5.8</td>
<td>9.4 ± 4.5</td>
<td>694 ± 352</td>
</tr>
<tr>
<td>Non-suicidal patient with major depression (n=13)</td>
<td>1.7 ± 1.4</td>
<td>6.9 ± 5.7</td>
<td>5.2 ± 4.7</td>
<td>355 ± 329</td>
</tr>
</tbody>
</table>

In comparison to depression, the biochemical and neuroanatomical changes induced by chronic stress have also been recognized to be associated with impulsive aggression in the causation of suicidal behavior. Functional neuroimaging studies of animals and humans have localized the effects of
chronic stress to regional areas of the brain. In animal studies, rats that have been subjected to chronic stress in the form of daily restraint showed significant dendritic atrophy, represented by decreased grey matter volume, in the medial prefrontal cortex and hippocampus [183-187]. The decrease in grey matter volume was the result of shorter dendritic branches and fewer dendritic branch points. In human studies using magnetic resonance imaging, patients with post-traumatic stress disorder showed significantly reduced grey matter volume in the medial prefrontal cortex, hippocampus, and amygdala when compared to controls [188-195]. Therefore, based on similarities between the neuroanatomical abnormalities associated with impulsive aggression and chronic stress, the hyperactivity of the HPA axis has been implicated as one of the neurological mechanisms responsible for the causation of suicide. As a result, the independent HPA activity induced by chronic stress and impulsive aggressive behavior may combine together in a synergistic manner to reach the characteristic levels of elevated HPA axis activity observed in individuals who attempted suicide.

The finding of a difference in serotonergic dysfunction and HPA axis activity between individuals with a history of suicide attempts and individuals without such a history suggests that suicidal behavior is strongly associated with impulsive aggression, depression, and chronic stress. However, the individual contribution from impulsive aggression, depression, and chronic stress to the level of serotonergic dysfunction and HPA axis activity in an individual has been
shown to be below the levels observed in suicide attempters. Therefore, the increased mortality rate associated with the combination of impulsive aggression and depression or chronic stress (Table 5) suggest that suicide most likely occurs when individuals with impulsive aggressive personalities suffer from a psychiatric illness, such as depression, or a significant life stressor, during which the individual contributions of serotonergic dysfunction and HPA axis activity combine together in synergistic effect to result in an enhanced level of serotonergic dysfunction and HPA axis activity that is similar to the levels seen during suicidal behavior. This enhanced level of serotonergic dysfunction and HPA axis activity is characterized by the same accompanying neuroanatomical abnormalities of volumetric reductions in the frontal lobe, hippocampus, and amygdala.

**Neuronal Plasticity and Suicidal Behavior**

Over the past two decades, studies in neuronal function have led to an evolving hypothesis that suggested a role of neuronal plasticity in the pathophysiology of suicidal behavior. The two processes that characterize neuronal plasticity, neurogenesis and neuronal remodeling, help the brain maintain homeostasis by regulating the proliferation, migration, and differentiation of new neurons into the neural circuits. For example, animal studies have shown experimental evidence of the role neurogenesis plays in formation and retention of memory. Animals irrigated to induce a state of
reduced neurogenesis showed deficits in spatial memory task performance and memory retention when compared to non-irradiated animals due to the fact that the irradiated animals had far fewer neurons to develop memory stability during the training period. [196]. The process of neurogenesis and neuronal remodeling are mediated by neurotrophic factors such as neurotrophins, glial cell-line derived neurotrophic factor, and neuropoietic cytokines. Neurotrophins, with brain-derived neurotrophic factor (BDNF) in particular, have been identified as the molecular mediators of synaptic and morphological plasticity responsible for the regulation, differentiation, and maintenance of functions in neurons [197-201]. For example, the involvement of BDNF in neuronal plasticity has been observed in the studies examining the visual cortex of animal subjects in vivo. During critical periods of development, when the visual input is blocked by monocular deprivation, the connectivity in the visual cortex of animals can be altered by rendering neurons nonresponsive to stimuli in the deprived eye. However, the local administration of BDNF into the visual cortex of the animal subjects during the critical period of development prevented the effects of monocular deprivation and maintained responsiveness in the deprived eye. [197] Therefore, in the event that neuronal plasticity becomes impaired, the brain fails to adapt and maintain homeostasis, thereby leading to the development of neuroanatomical abnormalities and functional impairments that contribute to the expression of suicidal behavior. Therefore, to evaluate the role of neuronal plasticity in the pathophysiologic mechanism of suicide, the involvement of BDNF will be
considered in the context of major risk factors of suicide, such as chronic stress and depression.

The effect of stress on the neuroanatomical structures related to suicidal behavior has long been recognized through animal and human studies. Subjects exposed to chronic stress are characterized by volumetric reductions of the frontal lobe, hippocampus, and amygdala [183-195]. These volumetric reductions, in the form of dendritic reduction and atrophy, indicate a failure of BDNF to maintain homeostasis. In animal studies, when rats were initially subjected to stress, a transient increase in BDNF expression was observed in structures of the brain, such as the hippocampus, pituitary gland, and hypothalamus, thereby suggesting a short-term preventative mechanism against dendritic damage by promoting cell growth and survival [202-206]. However, after the transient increase in BDNF expression, during acute and chronic stress the expression of BDNF mRNA is dramatically downregulated and accompanied by volumetric reductions in the hippocampus and frontal lobe [207-211]. In pharmacological tests in which rodents were injected with exogenous glucocorticoids, to mimic the effect of stress, the expression of BDNF was shown to be reduced in the hippocampus and frontal lobe [206, 212, 213]. The downregulation of BDNF mRNA is further supported by the observed increase in the BDNF receptor tyrosine kinase B (trk B) mRNA which serves as a compensatory adaptation to low levels of BDNF [214]. Therefore, by downregulating the expression of BDNF mRNA, stress has been shown to
contribute to the dendritic atrophy and decreased neurogenesis of areas in the brain associated with deterring suicidal behavior.

The relationship between BDNF and depression has also been demonstrated through brain imaging and postmortem studies of depressed patients. Volumetric reduction of the hippocampus, frontal lobe, and amygdala has been observed in patients with depression, indicating a failure of BDNF to maintain homeostasis. The decreased neuronal body size and glia cell density associated with the volumetric reductions, seen in depressed patients (Table 8), demonstrated the inability of BDNF to prevent atrophy or stimulate neurogenesis, which can be attributed to decreased levels of BDNF [215-217].

Table 8. Layer-specific mean neuron size and mean glial densities in the left rostral orbitofrontal cortex, left caudal orbitofrontal cortex, and dorsal lateral prefrontal cortex in psychiatrically normal controls and patients with major depression. Mean neuron size are described in 4 x (Area/Circumference) μm. Mean glial density are described in # of cells / (mm$^3$ x 10$^{-3}$ μm). Data adapted from Rajkowska G et al., 1999.

<table>
<thead>
<tr>
<th></th>
<th>Psychiatrically Normal Controls (n=12)</th>
<th>Mean Neuron Size</th>
<th>Mean Glial Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Rostral Orbitofrontal Cortical Region</td>
<td>12.9 ± 0.6</td>
<td>110 ± 17</td>
<td></td>
</tr>
<tr>
<td>Left Caudal Orbitofrontal Cortical Region</td>
<td>12.6 ± 0.6</td>
<td>105 ± 7</td>
<td></td>
</tr>
<tr>
<td>Dorsolateral Prefrontal Cortical Region</td>
<td>13.4 ± 0.9</td>
<td>100 ± 13</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Patients with Major Depression (n=12)</th>
<th>Mean Neuron Size</th>
<th>Mean Glial Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Rostral Orbitofrontal Cortical Region</td>
<td>12.3 ± 0.5</td>
<td>100 ± 17</td>
<td></td>
</tr>
<tr>
<td>Left Caudal Orbitofrontal Cortical Region</td>
<td>12.2 ± 0.5</td>
<td>91 ± 14</td>
<td></td>
</tr>
<tr>
<td>Dorsolateral Prefrontal Cortical Region</td>
<td>12.8 ± 0.6</td>
<td>92 ± 9</td>
<td></td>
</tr>
</tbody>
</table>
The usage of antidepressant treatments, however, can reverse these volumetric reductions and has been implicated in the modulation of BDNF expression. The chronic treatment using antidepressants has been shown to increase neurogenesis by increasing BDNF expression in the hippocampus and prefrontal cortex of rats (Figure 8) [218-220]. Rats chronically treated with duloxetine showed significantly higher levels of BDNF in the cerebral spinal fluid and prefrontal cortex when compared to control and vehicle-treated rats. Antidepressant treatment has also been shown to reverse the downregulation of neurogenesis caused by depression and stress. In studies using serotonin knockout rats to simulate the effects of depression, the chronic administration of duloxetine normalized or increased the naturally reduced expression of BDNF mRNA in the hippocampus and prefrontal cortex (Figure 9) [221, 222]. Similarly, in studies employing various forms of stress, the chronic administration of antidepressant drugs, such as venlafaxine and imipramine, was shown to enhance the rate of recovery in levels of BDNF when compared to the chronic administration of saline or nothing at all. [223, 224]. Based on these findings, the reduced expression of BDNF can be implicated in having a key role in the development of depression, which can eventually contribute to the suicidal behavior.
Figure 8. Total BDNF levels in the cerebral spinal fluid (CSF) and prefrontal cortex (PFC) of untreated rats (controls), vehicle-treated rats, and duloxetine treated (100 mg/kg/day) rats over the period of 14 days. Data adapted from Mannari C et al., 2008.
Therefore, due to the role of neurotrophins in the maintenance of neuronal functions, structural integrity of neurons, and neurogenesis, a detectable variation in the endogenous levels of BDNF may be of forensic significance for medical examiners. In consideration of the fact that depression and stress are recognized components of suicide and proven to influence the down regulation of BDNF expression, it would be rational to assume that a lower level of BDNF expression would be associated with suicide. Support for this association can be
found in post-mortem studies of suicide subjects. In fact, several studies have shown significant differences in mRNA and protein levels of BDNF and trk B within the brain of suicide victims in comparison to control subjects [225-227]. In each of these studies, the protein and mRNA expression of BDNF and trk B were determined and shown to be significantly decreased in the prefrontal cortex and hippocampus of suicide completers when compared to healthy controls (Table 9). The decreased protein level of BDNF was strongly correlated with its corresponding mRNA level, thereby suggesting less BDNF synthesis in the brains of suicide subjects. A study in particular, suggests the decrease in BDNF levels was only limited to areas of the brain associated with emotional control. In the study of BDNF expression in the prefrontal cortex, hippocampus, and entorhinal cortex of suicide subjects, significantly decreased levels of BDNF were only found in the prefrontal cortex and hippocampus (Figure 10) [227]. Furthermore, a study of patients with major depressive disorder has shown that levels of plasma BDNF were significantly lower in suicidal patients (430.5 ± 397.0 pg/mL) when compared to non-suicidal patients (875.80 ± 663.02 pg/mL) and controls (889.4 ± 611.3 pg/mL) [228]. The reduced plasma BDNF levels were no different between fatal and non-fatal suicide attempts, indicating an enhanced state of BDNF deficiency, compared to the BDNF deficiency of non-suicidal depressed patients, is required for suicidal behavior. Overall these findings suggest that BDNF is involved in the pathophysiology of suicidal behavior.
Table 9. Mean levels of messenger RNA (mRNA) and protein expression of BDNF and trk B in the prefrontal cortex and hippocampus of control subjects and suicide subjects. Data adapted from Dwivedi Y et al., 2003.

<table>
<thead>
<tr>
<th>Mean Values of BDNF and trk B mRNA and protein</th>
<th>Prefrontal Cortex</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>Control Subjects (n=21)</td>
<td>Suicide Subjects (n=25)</td>
</tr>
<tr>
<td>BDNF mRNA (attomole per microgram of total RNA)</td>
<td>606.8 ± 139.5</td>
<td>359.6 ± 96.7</td>
</tr>
<tr>
<td>BDNF protein (arbitrary units)</td>
<td>1.61 ± 0.39</td>
<td>0.91 ± 0.25</td>
</tr>
<tr>
<td>trk B mRNA (attomole per microgram of total RNA)</td>
<td>1168.3 ± 322.3</td>
<td>704.5 ± 225.7</td>
</tr>
<tr>
<td>Full length trk B protein (arbitrary units)</td>
<td>1.29 ± 0.31</td>
<td>0.87 ± 0.19</td>
</tr>
<tr>
<td>Truncated trk B protein (arbitrary units)</td>
<td>1.36 ± 0.32</td>
<td>1.44 ± 0.27</td>
</tr>
</tbody>
</table>
Figure 10. Mean values of BDNF protein in the hippocampus, prefrontal, and entorhinal cortex from non-suicidal controls and 3 groups of suicide victims.

Figure adapted from Karege F et al., 2005.
4.3. Consideration of Hypotheses of Suicide Pathophysiology and Suggested Guideline for Classifying Manner of Death

When comparing the viability of the two hypotheses of suicide pathophysiology, the similarity in neuroanatomical abnormalities and functional impairments, in relation to depression, chronic stress, and postmortem studies, suggests the possibility of the two hypotheses being interrelated and interconnected. The enhanced state of BDNF deficiency observed in suicidal patients of major depressive disorder, compared to non-suicidal patients, may be explained by the increased reactivity and response to stress and depression by individuals with impulsive aggressive personality traits. Likewise, the increased response to stress and depression in suicidal individuals with impulsive aggressive personality traits may be attributed to the enhanced susceptibility to serotonergic dysfunction and HPA axis activity generated from the failure of BDNF to maintain homeostasis. Therefore, in consideration of formulating a comprehensive method to differentiate biological markers of suicidal behavior from the usual neuroanatomical alterations associated with depression and chronic stress, the significant differences observed in suicidal subjects from the two hypotheses of suicide pathophysiology will be integrated into one diagnostic guideline. Based on the experimental evidence from multiple studies, these significant differences include an enhanced state of serotonergic dysfunction, HPA axis activity, and BDNF deficiency. The forensic applicability in detecting
and measuring these differences in post-mortem samples will be discussed in the paragraphs that follow. The guideline for the new proposed form of diagnosing suicidal behavior is an adaption of autopsy practices and procedures from studies that have shown successful extraction and quantitation of molecules of interest. The guideline is not meant to replace the findings from a scene investigation nor background history. Taken into consideration independently, the results from this guideline will only suggest if the decedent was in a suicidal state of mind.

**Postmortem Brain Collection and Processing**

Upon reaching the stage of the autopsy to examine the brain, the hair should be separated and an incision should be made, from ear to ear, across the vertex of the scalp. The scalp should then be reflected and another incision should be made to reflect the temporalis muscle. Using an oscillating saw, the calvarium should be cut and the skull cap should be detached from the dura. Next, the superior longitudinal sinus is to be opened and the dura along the cut on the skull should be cut and reflected toward the midline to expose the brain. At this point, the brain is to be inspected for any trauma or anatomical abnormalities. The frontal poles are then to be retracted and the anterior attachments of falx cut. Afterwards, the olfactory bulbs are to be elevated and the brain retracted to expose the optic nerves, carotid arteries, and any other attached nerves which are to be cut. The brain is then to be retracted medially,
so the tentorial attachments along the petrous ridges can be cut, and then retracted posteriorly, so the spinal cord and any remaining cranial nerves or vertebral arteries can be cut as distally as possible. Finally, the cerebellum and brainstem is to be retracted in order to cut any remaining dura that may be keeping the brain still attached to the skull [229].

Following the removal of the brain, sections of the prefrontal cortex and hippocampus are to be dissected from the right hemisphere of the brain. The right hemisphere of the brain was selected because structural abnormalities in the area have been shown to be associated with antisocial, violent, and suicidal behavior [230, 231]. The prefrontal cortex is to be considered the gray matter from the most anterior 1-cm coronal slice of the cortex and a block of tissue is to be dissected from Brodmann area 9 according to a Brodmann atlas. This isolated piece of tissue is to be stored at -80 °C until finer dissection is required. When the time comes for finer dissection, the tissue is to be thawed from -80 °C to -20 °C, placed onto a glass surface with dry ice, and dissected to isolate 0.5- to 1-cm coronal slices of gray matter without the incorporation of any white matter. The distinction between gray and white matter is important during dissection because gray matter is composed of cell bodies while white matter is composed of nerve fibers. For the hippocampus, 0.5-cm coronal slices of gray matter, without the incorporation of white matter, are to be dissected from the middle of the hippocampus and should include the dentate gyrus and areas of CA1-4. If these isolated samples of tissue are not to be used immediately, they should be stored...
at -80°C. When the time comes to test the tissue samples, the individual pieces from the prefrontal cortex and hippocampus should be homogenized separately with a tissue grinder using appropriate buffer solutions. Although the amygdala has been implicated in serotonergic dysfunction and HPA axis activity, studies of the amygdala in the context of suicidal behavior is limited therefore the analysis of the amygdala will be excluded from this guideline. These procedures for analyzing the prefrontal cortex and hippocampus were adapted from studies that have successfully quantitated serotonin, BDNF, and trk B in suicide victims. Factors such as post-mortem interval, sex, age, and brain pH were not shown to have any significant effect on the outcome the analysis and were excluded during consideration.

**Quantification of Serotonergic Dysfunction**

As the studies have shown, serotonergic dysfunction is associated with low CSF concentrations of serotonin and serotonin metabolite 5-HIAA, elevated dopamine levels, low prolactin levels during a fenfluramine challenge, volumetric reduction of the prefrontal cortex, a reduced number of presynaptic serotonin receptors, and an increased number of postsynaptic serotonin receptors. However, the quantitation and comparison of all these differences may not be a viable option during an autopsy exam. CSF concentrations of serotonin, 5-HIAA, and dopamine are known to fluctuate throughout the day and are immediately increased by alcohol, drugs, and antidepressant use. In deaths due to
drug/ethanol overdose, the CSF concentrations of serotonin, 5-HIAA, and dopamine would be skewed and an inaccurate representation of the level of serotonergic dysfunction. The alternative options of measuring prolactin levels during a fenfluramine challenge or measuring the volumetric reduction in the prefrontal cortex are also unfeasible options since the tests are required to be performed in vivo. The only potential candidates able to represent serotonergic dysfunction are the reduced number of presynaptic serotonin receptors and the increased number of postsynaptic serotonin receptors. Unlike CSF concentrations of serotonin and 5-HIAA, serotonin receptors are less susceptible to change in the presence of drugs and alcohol and offer a more reliable representation of the degree of serotonergic dysfunction. Therefore, mRNA and proteins levels of presynaptic serotonin transporter sites and post synaptic 5-HT$_{2A}$ and 5-HT$_{1A}$ receptors should be quantified using competitive reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot analysis, respectively, and compared to values from depressed patients and healthy controls. An individual in a suicidal state of mind would show significantly decreased mRNA and protein levels of presynaptic receptors along with significantly increased mRNA and protein levels of postsynaptic receptors in the prefrontal cortex, when compared to depressed patients and healthy controls.
**Quantification of HPA Axis Hyperactivity**

In contrast, the hyperactivity of the HPA axis is associated with low CSF concentrations of serotonin, high CSF concentrations of dopamine, epinephrine and norepinephrine, high cortisol response during a dexamethasone and corticotrophin releasing hormone challenge, and volumetric reduction of the prefrontal cortex and hippocampus. Similar to serotonergic dysfunction, the CSF concentrations of serotonin, dopamine, epinephrine, and norepinephrine during HPA axis hyperactivity are affected by alcohol, drug, and antidepressant usage and are unreliable markers of the degree of HPA axis hyperactivity. In addition, in cases of chronic stress, the concentrations of dopamine, epinephrine, and norepinephrine are constantly changing as they shift from elevated levels to decreased levels as the monoamine stores become depleted due to the prolonged HPA axis activity. The alternative options of measuring cortisol response during a dexamethasone and corticotrophin releasing hormone challenge or measuring the volumetric reduction in the prefrontal cortex and hippocampus are also unfeasible options since the tests are required to be performed in vivo and because there are currently no viable means to measure this difference during autopsy.

**Quantification of BDNF Deficiency**

BDNF deficiency, on the other hand, has been associated with volumetric reductions of the frontal lobe and hippocampus, low levels of BDNF in the blood,
and decreased protein and mRNA expression of BDNF and trk B in the prefrontal cortex and hippocampus. Similar to the other models, the measurement of the volumetric reductions of the frontal lobe and hippocampus is an unfeasible option since the procedure requires it to be performed in vivo. Unlike with monoamines, the usage of alcohol, drugs, and antidepressants does not immediately alter the levels of BDNF in the plasma, serum, or platelet, therefore the low concentrations of BDNF in the blood may hold potential in serving as a marker of BDNF deficiency. Strenuous exercise has been shown to immediately increase the levels of BDNF in the blood, but that level of physical activity is not commonly associated with casualties due to drug/ethanol overdose [232, 233]. Decreased protein and mRNA expression of BDNF and trk B in the prefrontal cortex and hippocampus may also serve as potential markers of BDNF deficiency. However, caution should be exercised when dealing with drug/ethanol overdoses that involve chronic abuse; studies have shown significant reductions in serum BDNF levels in patients with alcohol dependence [234, 235]. Therefore, to diagnosis an enhanced state of BDNF deficiency that is suggestive of suicidal behavior, the serum BDNF levels should be compared to patients with depression and healthy controls. In addition, the mRNA and protein levels of BDNF and trk B receptors should be quantified using competitive reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blot analysis, respectively, and compared to values from depressed patients and healthy controls. An individual in a suicidal state of mind would show significantly decreased serum BDNF levels and
significantly decreased mRNA and protein levels of BDNF and trk B receptors in the prefrontal cortex and hippocampus, when compared to depressed patients and healthy controls [225-228].

**Quantification of Protein Levels**

Protein levels are to be measured using the Western blot technique. Western blots are routinely used for analyzing protein mixtures and allows for the determination of the molecular weight and relative quantity of a specific protein. The general process begins with the separation of macromolecules using gel electrophoresis and is followed by the transfer of the separated protein pattern onto a second matrix which is typically a membrane. The surface of the membrane is then treated with generic protein to prevent any nonspecific binding of antibodies to the membrane and an enzyme-linked antibody is added to bind to the specific protein of interest. Upon the addition of a suitable substrate, the enzyme linked to the antibody will produce a product that can be detected and measured.

The general steps to quantify protein levels have been summarized in the figure below (Figure 11).

Homogenization of brain tissue

↓

Determination of protein concentration using 1-dimensional
Western blot
↓
Gel electrophoresis
↓
Protein pattern on gel transferred to membrane
↓
Incubated with primary antibodies for target proteins (5-HTT, 5-HT2A, BDNF, trk B)
↓
Membrane washed
↓
Incubated with β-actin antibodies (used as a loading control)
↓
Membrane washed
↓
Incubated with secondary antibodies for primary antibody
↓
Membrane washed
↓
Membrane developed with chemiluminiscent reagents and radiographic film
↓
Determination of the optical densities of target proteins in comparison to a reference protein (β-actin)

Figure 11 - Flowchart summary of steps taken to quantify protein levels.

Quantification of mRNA levels

mRNA levels are to be measured using the competitive reverse transcriptase-polymerase chain reaction. Competitive RT-PCR is typically used to measure the total amount of a specific mRNA sequence in a sample. The process involves combining dilutions of synthesized RNA sequences, which serve as internal standards and are identical in sequence but shorter in length
than the endogenous mRNA target, with sample RNA replicates before both are co-amplified together. The synthesized RNA sequence is capable of being amplified by the same primers and with the same efficiency as the target endogenous target, therefore it serves to compete with the endogenous mRNA transcript during the amplification process. Quantitative measurements of target mRNA can then be made by comparing the PCR product signal intensity of the endogenous target with the concentration curve generated by the synthetic competitor RNA sequence.

The general steps to quantify mRNA levels have been summarized in the figure below (Figure 12).
comparing signal intensity with internal standard

**Figure 12 - Flowchart summary of steps taken to quantify mRNA levels.**

5. CONCLUSIONS

Using an in-depth literature review, the issues contributing to the classification of undetermined manner of death were identified and addressed. A new guideline, based on scientific studies from the field of neurobiology, was formulated to propose an alternative method of diagnosing suicidal behavior in order to differentiate between the accidental and suicide manners of death in undetermined deaths from drug or ethanol overdose where medical and investigational findings are insufficient. The new method of testing differentiated between the classification of an accident and a suicide by proving the decedent was in a suicidal state of mind, characterized by specific neuroanatomical abnormalities observed from post-mortem studies. The predisposition to suicidal behavior is established by comparing the degree of serotonergic dysfunction, HPA axis activity, and BDNF deficiency observed in an individual to the levels observed in patients with depression and healthy normal controls. Based on the guideline, an individual in a suicidal state of mind would show significantly decreased mRNA and protein levels of presynaptic receptors along with significantly increased mRNA and protein levels of postsynaptic receptors in the
prefrontal cortex, significantly decreased serum BDNF levels, and significantly decreased mRNA and protein levels of BDNF and trk B receptors in the prefrontal cortex and hippocampus, when compared to patients with depression and healthy controls. The values from suicide subjects were not included for comparison because statistical analysis in studies had shown that all the values from suicide subjects were significantly lower than the patients with depression and healthy controls, therefore any range of determined values which are significantly lower than the patients and healthy controls could suggest a similar pattern to suicide.

The burden of the financial costs associated with performing the quantitation of both mRNA and protein levels of BDNF, 5-HTT, and 5-HT₂A receptors, however, may not be welcomed by some medical examiners. The equipment and reagents required to quantitate mRNA and protein are costly and may exceed the office of the medical examiner's financial capability. Currently, the cost of autopsies are covered by state taxes and the additional cost for equipment, training, and reagents, especially in smaller states with a lower mortality rate, would be hard to justify. In addition, the extra labor associated with the testing would tax the resources and manpower of many offices and create an even larger backlog of items that need to be processed. Although these are justifiable concerns, it must be taken into consideration that the new form of testing is intended to resolve a very specific scenario which could not be resolved using the current methods of testing. Depending on the frequency in which
medical examiners encounter these scenarios and the amount of third party influence, for some medical examiners, the benefits of resolving the classification may outweigh the costs.

In order to reduce the financial burden imposed by performing both RT-PCR and Western blotting to quantitate the mRNA and protein levels in BDNF, trk B, 5-HTT, and 5-HT$_{2A}$ receptors, it is suggested that the tests be separated into presumptive and confirmatory testing. The levels of serotonin presynaptic and postsynaptic receptors, 5-HTT and 5-HT$_{2A}$ respectively, have been shown to be altered by the chronic use of SSRIs (selective serotonin reuptake inhibitors) [236], while the levels of BDNF and trk B showed no significant difference [230]. Therefore, since the quantitation of mRNA and protein levels of 5-HTT and 5-HT$_{2A}$ is susceptible to the chronic use of SSRIs, the quantitation of mRNA and protein levels of BDNF and trk B should serve as the confirmatory diagnosis. By separating the testing into presumptive and confirmatory testing, the amount of testing and resources required can be reduced to a financially manageable level.

In comparison to the current methods used to classify manners of death, there are many advantages in adopting this guideline to classify undetermined overdose deaths. The main advantage is the ability to produce empirical evidence to combat the innate subjectivity of the determination process when medical and investigational evidence is lacking. Concerned third parties would no longer be able to threaten medical examiners with legal action if a death is classified as a suicide because the determination would be based on scientific
and medical proof. In addition, the new testing method would be able to resolve many of the scenarios that cause ambiguity between an accident or suicide classification by being based on something that not readily alterable. Scenarios where critical items of evidence go missing or where third parties provide false information would not affect the classification as it would in the usual manner. The levels of mRNA and protein expression of serotonin receptors, BDNF, and trkB in the brain are not easily manipulated and even with the use of drugs, alcohol, and anti-depressants, a long period of usage is required before any noticeable changes in levels are observed.

From a forensic and legal perspective, the new testing method is ideal for maintaining chain of custody. The evidence, which is the brain of the decedent, is protected by the skull, making it difficult to contaminate or destroy, and only trained personnel would be able to harvest the brain. In addition, the brain is located far from the effects of decomposition or digestive fluids. In fact, studies have also shown that effects such as age, postmortem interval, sex, and brain pH have no significant effects on the expression levels of BDNF and trkB [225].

Although this new method of testing may potentially allow medical examiners to determine and assign an accidental or suicide manner of death in undetermined overdose deaths by drug/ethanol overdose when medical and investigational evidence is inconclusive, as well as reestablish their professional authority, caution must be exercised in accepting the guidelines in its current state. The proposed state of enhanced serotonergic dysfunction, HPA axis
activity, and BDNF deficiency that is indicative of suicidal behavior was formulated from a limited number of studies. This may be due to the difficulty in procuring samples for study, but nonetheless the observations from a larger number of studies should be incorporated into the guideline. In addition, although the studies have also shown that effects such as age and postmortem interval did not significantly affect the expression of BDNF and trk B, the validity of the statement is also challenged by the limited pool of subjects from which the information was taken. BDNF has been known to decrease with age and the postmortem interval in the studies were limited to a maximum of 36 hours; therefore, areas of future research could include studying the effects of age from a larger population and the effects of postmortem interval from a longer period of time. In addition, this guideline did not take in consideration the effect that sex has on the expression of serotonergic dysfunction, HPA axis activity, and BDNF deficiency. The effect of gender has been shown to play a role in the BDNF signaling and expression of 5-HT_{1A} receptors [237-239]. By incorporating the findings in these areas of study, this guideline may gain further support and develop into an accepted method of identifying a predisposition towards suicidal behavior.
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This paper examines the neuroanatomical markers observed in suicidal behavior and suggests a method to incorporate these markers in differentiating between an accidental and suicide manner of death in cases of drug/ethanol overdose.
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