Chapter 2

The Diaeventology of Anxiety Disorders

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Additional information is available at the end of the chapter

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Abstract

Anxiety is a crippling neuropsychiatric condition that encompasses a complex endophenotypic network of genetic, immunological, epigenetic, and metabolic mechanisms, interacting with the environment. A new approach to complex biological systems, including mental states and their neurological correlates, is diaeventology, a paradigm that exposes the event ontologies of these biomolecular/cellular mechanisms. General anxiety disorder has been studied in subclinical and clinical research settings where evidence is obtained for a longitudinal patterning of chronic and episodic and often increasingly stressful life events that provide the etiology and development of the pathology. Early events that involve brain oxygen deprivation coupled with carbon dioxide abundance are linked to biochemical and epigenetic processes associated with anxiety instantiation. A verified analysis of the current evidence suggests a neuroimmune mechanism that aligns with stress pathophysiology and epigenetic re-tailoring of key genomic loci that inappropriately compensate and misdirect biological defense mechanisms toward central nervous system dysfunction presenting as anxiety disorders.

Keywords: anxiety, diaeventology, genomics, epigenomics, neuroimmune

1. Introduction

Anxiety is associated with psychological states that consubstantially ignite an imbalance with the neuroimmune system. For example, cancer anxiety has been associated with a decrease in natural killer (NK) cells [1]. It is well established that care-givers of the terminally ill score higher than non-family members on the Hospital Anxiety and Depression Scale (HADS); but over time, this value decreases as the family member adapts to the worsening condition of their loved one [2]. These aspects of anxiety obtain neural correlates that are both endocrine stress hormone-linked and epigenetic in origin, with links to the immune system. Combined

with an environment of stress, immune responses can epigenetically alter the expression of genetic loci and potentially modify those genes directly. This association of stress with later anxiety manifestation may have deeper roots going back to in utero events affecting the neuroimmunoepigenome of the fetus.

The interplay of organisms is the macrocosm, but it also appropriately describes the human body and overall stress imposed by the microbiome, invading pathogens, autoimmunity, cancer, autophagy, and senescence. The development, differentiation, and the signal transduction cascade network, including neuronal action potentials and endocrine mediation, also compose an opposing three-dimensional trigonal plane where the central element is the homeostasis of the existing individual. Indeed, learning and the accumulation of memories and knowledge are all part of a massive internal interactome that can be understood relative to advantage, vectorial control, and constant failure. Anxiogenesis can arise from perturbations to this system and manifest at the physiological and neuropsychological level.

There is a natural-native system that encompasses all of the features describing this neuropsychiatric interactome and its axis is the immune system. Thus, the immune system has two roles in the human body. One is for defense plus offense, and the other, in conjunction with epigenetic mechanisms, generates the existing individual. This is a perpetuating neural network that can learn, via attention and ascent to stress, to function within the world. Such a biologically adaptive phenomenon is accomplished via homologous recombination of variable regions of both the immunoglobulin family and the T-cell receptor in concert with chromatin remodeling, the histone code, and both the acetylome and methylome of cohering DNA. If there is a link between the mind and the body, at least one component is physical. This connection might be the molecular and cellular adaptive immunological interactome that serves to generate neural tracts according to developmental, endocrine, and peripheral stimuli while maintaining repair processes in the central nervous system (CNS) by using the complex interactions between microglia and neurons. Neural networks are processes, becoming eventual in nature, as contrasted with a substance ontology. The pattern of events appears as energetic fields rather than particles of matter, much like chemical bonds. An event ontology that reaches across the neural network of genetic and environmental interaction is articulated and synthesized through epigenetic modifications of the classical immune system, thus creating a responsive plastic and elastic memory field capable of learning, ideation, imagination, and understanding through time. This diaeventome involves the central existing individual agentically interacting through the events obtained across the inherited genome, the changing environment, and the transcendental immunoepigenome through time.

Figure 1 represents the current model.

This chapter will employ my new paradigm of diaeventology to introduce and instantiate the neural correlative endophenotype linked to the psychiatric condition general anxiety disorder (GAD).

Diaeventology (dialectical event ontology) is an adaptational, processive scientific accounting of the physiologico-rational human condition through cellular and molecular event ontology.

Its biological mechanism of action, linking pathopsychological states like GAD to biochemical pathways, incorporates the immuno-epigenomic induction of neural, endocrine, and

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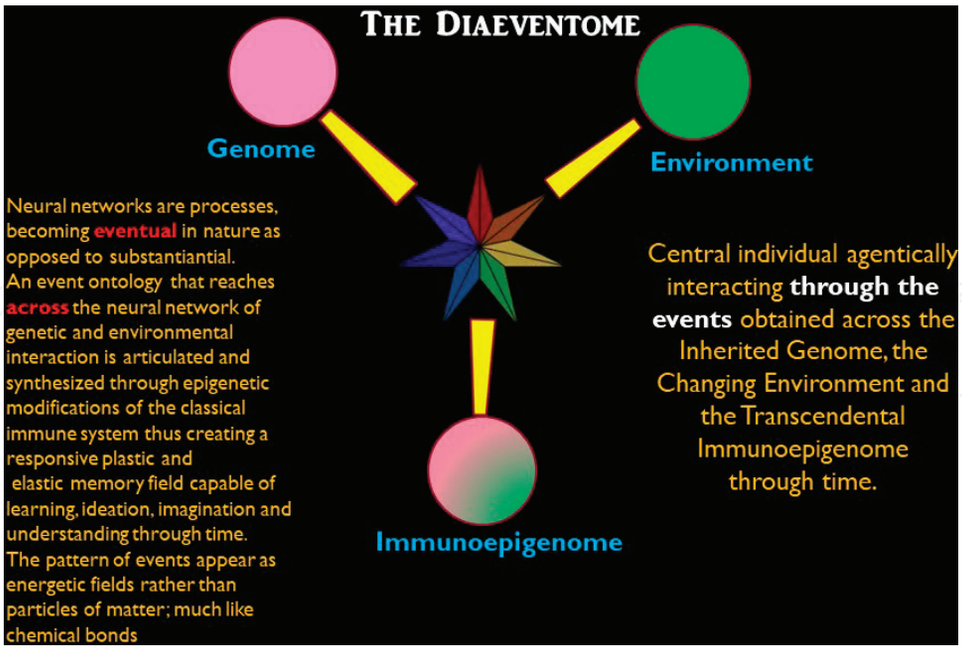

Figure 1. The diaeventome paradigm.

metabolic response to the micro- and macro-environment through classical constitutive-surveillance and acquired-effector cellular and humoral defense stratagems using reversible covalent modification and hydrophobic interactions of nucleic acids, proteins, and lipids.

Thus, I will explore how diaeventology offers a general molecular system theory for a free will-driven/agency-based individual adaptation and a knowledge-acquiring physiologico-rational mechanism that better explains the core event ontology of human existence including health and well-being.

2. Diaeventological sources of GAD

During early stages of embryo implantation, there is a suggested suppression of the immune response; yet, there are many immune systems at play. These include NK cells. NKs are innate immune cells that require no secondary or tertiary recombination and adaptation to kill target cells upon antigen presentation as with Major Histocompatibility Complex (MHC) class I-held antigens [3]. This allows NK cells to degranulate and release cytotoxic substances directly into targeted cells for destruction. There is a pull back, or switch, that may involve mesenchymal stem cells signaling through interferon that regulate and therefore license and delicense the NK killing based on chemokine reception and a global on/off switch [3].

A recent report catalogs some of the descriptors of immune surveillance in the uterus. In this chapter, it is reported that macrophages, NK cells, and T cells are found in the human decidua [3]. Over 70% of the detectable immune cells are NKs and the rest are mostly macrophages with small percentages of dendritic cells. They also summarize from the literature that no B or plasma cells can be detected. However, the remainder of the immune cell population is of T-cell lineage.

Of interest to the argument that the diaeventome drives an epigenetically modifiable immune-based regulation of global physiological and pathophysiological consequence is that the ablation of NK cells prevents trophoblasts from obtaining endometrial vascularity. This results in spontaneous abortion. Whether this phenomenon is associated with controlled cell destruction or chemokine/cytokine-mediated signaling, leading to reprogramming of gene expression, is not yet clear, but it does suggest that NK cells may be necessary for in utero trophoblast invasion. Since dendritic cells play a key role in communication between the constitutive and adaptive immune response, it is of further note that loss of uterine Dendritic Cell (DCs) blocks decidual maturation and blastocyst implantation [3].

Stress can induce epigenetic changes to loci that control the expression of RNAi production. RNAi epigenetics involves the production of interfering RNA species and thus prevents target mRNA expression. This removal of target mRNA can have global or specific effects on gene expression including those involved in psychiatric and mood disorders [4].

Even though chronic psychological and social stress has been implicated in anxiety disorders, the mechanism for how social defeat and worrying can be linked to genomic or epigenomic phenomena has been difficult to track.

Recently, it was reported that chronic stress in a murine model was targeting an RNASE II enzyme complex (DROSHA subunit) via differential hypomethylation at that locus. A decrease in methylation suggests there is a concomitant increase in the non-specific expression of the target gene, and in this case, it would mean an increase in RNAi-mediated epigenetic ablation of gene expression [5].

In a rat model, pro-inflammatory CNS-localized M1 type microglia are induced by cumulative unpredictable mild stress (CUMS) within the Hypothalamic Pituitary Adrenal (HPA) axis [6]. This resulted in the expression of pro-inflammatory tumor necrosis factor (TNF)-α, interferon (INF)-γ, interleukin (IL)-1β, and IL-17 cytokines while simultaneously reducing the production of the anti-inflammatory IL-4, IL-10, and IL-13 cytokines typically associated with the regulatory M2 microglial lineage [6].

Macrophages are classified into inflammatory or anti-inflammatory. Inflammatory macrophages differentiate in response to microbial and tumor antigens and interferon γ by producing pro-inflammatory cytokines at the site of nascent infection and cancerous lesions while anti-inflammatory macrophages differentiate via signaling by glucocorticoids or anti-inflammatory (type II) cytokines like IL-4, IL-13, and IL-10 where they promote TH2 immunity and mediate tissue remodeling, wound healing, and immune modulation [7].

The cytokines IL-4 and IL-13 drive anti-inflammatory macrophage polarization through the IL-4 receptor alpha chain (IL-4Rα), and anti-inflammatory polarization is also promoted by activation of several master regulators, including signal transducer and activator of transcription 6 (STAT6), Krüppel-like factor 4 (KLF4), and interferon regulatory factor 4 (IRF4) [7].

Diet and nutritional life style choices likely modulate macrophage polarization and, by inference, the inflammatory response associated with anxiety disorder. Bioenergetic reprogramming is associated with this mechanism wherein the inflammatory macrophage cell type is fueled by

aerobic glycolysis and can be triggered by the bacterial antigen LPS ± the pro-inflammatory cytokine IFN-γ. Within the anti-inflammatory lineage, IL-4 induces the expression of PPARγ which in turn transcriptionally activates the urea cycle enzyme arginase 1 (Arg1) and the β-oxidation of fatty acids along with electron transport chain/oxidative phosphorylation gene expression and an increased capacity for mitochondrial biogenesis [7]. To fuel the anti-inflammatory bioenergetics, IL-4 also induces expression of CD36 which acts as a membrane receptor for circulating LDL and very low density lipoprotein (VLDL)-rich triacylglycerol (TAG). Finally, the unloading of triacylglycerol (TAG) and associated fatty acid hydrolase activity is linked to fatty acid oxidation, thus completing the anti-inflammatory polarization phenotype [7].

Recently, a macrophage-specific cytokine has been linked to anxiety. The macrophage migration inhibitory factor (MIF) is a pro-inflammatory macrophage-specific cytokine that is active in the HPA axis and characterized haplotype variants of that gene were linked to diminished expression and lowered adolescent anxiety disorder [8]. MIF has been linked to the recruitment of natural killer T cells via an IFN-γ gradient in skin lesions, thus suggesting a similar role in causing the migration and stimulation of inflammatory leucocytes in the HPA axis [9]. Indeed, MIF has been implicated with this dual cytokine/chemokine role in a large cluster of inflammatory diseases, thus suggesting a global immunopathological association of macrophages and other leucocytes in neuropsychiatric disease [10]. A previous report suggested that deletion or pharmacological inhibition of MIF biological activity in the hippocampal gyrus of mice resulted in anxiety-like behavior and this was correlated with a lack of neurogenesis in the region [11].

Combined, this evidence on macrophage switching, inflammation, and neurogenesis (thus targeting the canonical HPA axis) all point to a diaeventological progression of both environmental and genetic plus epigenetic event ontologies that instantiate a temporal link to anxiety disorders.

3. Acquired neuroimmune responses and GAD

The serotonin transporter (HTTPLPR) has been linked to depression and GAD in human populations. The short allele of the HTTPLPR gene was associated with these neuropsychiatric disorders although whether there was a hypo- or hyper-HPA axis effect depended upon the cohort population under study including parameters age, race, and gender [12]. However, this is not necessarily ambiguous, since the downstream processing of serotonin binding to its receptor is complicated by the level of allele-specific HTTPLPR-mediated translocation, availability of serotonin, plus the receptor subtype, and ultimate release of glucocorticoid via the HPA axis [12].

There are serotonin receptors on macrophages, monocytes, and lymphocytes, and these subpopulations interact to mediate inflammatory responses leading to HPA axis activity [13].

Serotonin has been associated with a blockade of the antigenic determinate capacity of macrophages via IFN-γ, thus diminishing the suppression of NK cells and therefore enhancing their potential cytotoxic function on host cells [13].

For their part, NK cells contribute to inflammation via their frank cytotoxicity, thus releasing potential activating antigens of pathogenic origin. NK cells also establish and maintain the "cytokine storm" which lays out the persistence and maturation of the local inflammatory response [14]. Certain non-cytotoxic clones of NK cells over-express high IFN-γ while others, that are manifestly cytotoxic, produce negligible amounts of IFN-γ. A third sub-population of NKs weighs in with intermediate characteristics [14].

Upon signaling-based activation, NKs kill their target via direct cellular contact involving either secretory lysosomal cytotoxic perforin and granzymes or deployment of the "death receptors and ligands" such as FasL and TRAIL, which mediate target cell apoptosis [14]. This shift to NK cell plenary function can mediate effects in the HPA that could result in either attenuation or enhancement of glucocorticoid signaling that would impact GAD and Major Depressive Disorder (MDD).

While these interacting immune responses maintain a metastable inter-uterine environment, the successful development of the fetus requires a perpetual modification of the mechanism that must deliver a balance between surveillance of potential toxic stress metabolites and pathogens on one hand, and the tolerance of the developing baby on the other.

Consequently, the presence of immune cells at the implantation site is not associated with a response to the "foreign" fetus but to facilitate and protect the pregnancy. Therefore, it should be theorized that the immune system at the implantation site is not suppressed; on the contrary, it is active and functional and is carefully controlled via real-time procession of gene expression—controlled by the developmental program between mother and fetus and the epigenetic modifications that are necessary for signal response (including stress) from the entire biological system including the external environment.

4. Epigenetics of the early uterine environment and potential for GAD imprinting

Anxiety disorder is clearly associated with biochemical and cellular phenomena. The interactions between the genome and the environment are well described and paradigmatic in the biomedical literature. However, the link to behavioral and neuropsychiatric disorders has been more recent and the inclusion of the epigenome is particularly compelling.

Genetic and "epigenetic" mechanisms shape biological activity and can respond negatively to produce the pathophysiological state. While the mammalian genome establishes the template for empirically discernable developmental and behavioral patterns, a more complex and variable phenomenon helps to produce the final phenotype. This latter "epigenetic" mechanism has increasingly become the subject of developmental and cell biology, gene expression, and disease. The biochemistry of epigenetics involves several covalent modifications of nuclear chromatin as well as post-transcriptional gene silencing [15].

Among these modifications is the methylation of the C5 atom on cytosine residues found in certain canonical CpG islands associated with promoter elements. Methylation, acetylation,

ubiquination, and phosphorylation of cohering histones and the processing of double-stranded RNA in the generation of siRNA are epigenetic phenomena involved in the modulation of gene expression. The mechanisms of these epigenetic phenomena have been described and they include the activities of methyltransferases, acetyltransferases, kinases, phosphatases, demethylases, deacetylases, E3 ubiquitin ligases, and RNase enzymes [16]. The substrates for these reactions are either chromatin or in the case of the RNase activities, double-stranded mRNA. S-adenosyl methionine (SAM or AdoMET) is the recognized nuclear methylation agent, deriving the methyl group from folic acid derivatives. Acetyl CoA is used in acetylation of chromatin-associated histones in the process of chromatin remodeling which generally enhances gene expression downstream from ligand/receptor-mediated activation of the complex which may be in association with the nuclear ubiquitin/proteasomal pathways. Nuclear-associated posttranslational modifications (such as acetylation) of histone carboxyl termini clearly alter chromatin structure and function [16]. The major effect is a pronounced change in the physical-chemical accessibility of DNA-binding proteins to unwind the double helix and potentiate the transcription to RNA. These covalent modifications are at least conceptually reversible, but often, they can lead to a complete removal of histones from the chromatin complex, thus inducing for a time in the cell cycle, uncontrolled constitutive gene expression. Indeed, while methylation tends to dissociate histones from the chromatin complex, demethylation tends to favor non-transcribable chromatin rearrangement although this leaves open the potential for acetylation which often promotes chromatin remodeling and gene expression [16].

Besides the specificity of the methyltransferases and acetyl transferases on certain histone residues (typically LYS), there is also a specificity at the amino acid sequence level. To generate changes in reactivity of chromatin to remodeling, only certain covalently modified histone amino acid residues play a role. The discrete biochemistry of these epigenetic modifications are lysine methylation, acetylation and ubiquination, serine phosphorylation, and arginine methylation. All of these modifications have been observed by superimposition of the diet (see below). The point is that, these covalent modifications effect DNA accessibility to various proteins and they alter protein:protein interactions among chromatin-bound histones and other polypeptides [16].

The "histone code" hypothesis asserts that covalent modification of chromatin-bound histones is communicated to a host of nuclear proteins to provide a directive for discrete chromatin molecular dynamics and gene expression control. The theory suggests that other proteins and protein complexes can distinguish and indeed interpret histone modifications. Communication of the histone code to the nuclear machinery of transcription ultimately controls gene expression or silencing, heterochromatin formation, DNA replication, and even chromosome segregation [16]. All of these mechanisms play a diaeventological role in neuropsychiatric states such as anxiety.

Most if not all of these epigenetic modifications are heritable changes in gene expression. Even though DNA sequence modification does not generally occur, there are reports where amplification of nucleotide repeats can be proximal to DNA methylation. Whether or not this is a common phenomenon in acquired epigenesis may be significant in neuropsychiatric disease. What is clear is that many developmental disorders as well as cancer, age-related illnesses,

and various brain disorders are linked to changes in DNA methylation. Epigenetic modifications (especially DNA methylation) provide a fine tuning on gene expression. Induced hypermethylation by xenobiotics as well as hypomethylation are linked to these diseases [16].

Besides involvement in various diseases, epigenetic phenomena are developmentally programmed. As such, epigenetic control over gene expression and cell differentiation as well as tissue formation and neurogenesis has been extensively reported. Epigenetics also plays a major role in immune response. In fact, mechanisms including CpG methylation and various histone modifications are basic biochemical phenomena regulating the mammalian immune response. Chromatin remodeling as well as the cohering epigenetic control over transcriptional processes have been shown to help regulate cytokine expression and secretion as well as antigen processing and T-cell differentiation. In this way, environmentally controlled epigenetic mechanisms as well as the methylation state of the immune cell chromatin are involved in the differentiation of T helper cells which in turn activate both T-cell and B-cell-mediated acquired immunity [17].

Since it is well documented that epigenetics plays a role in emotions and behavior, the link between immune response and emotions such as anger, depression, and anxiety has both a strong theoretical and empirical basis. I have previously discussed the role of cytokines in inducing the RAGE circuitry [15]. As it turns out, transcriptional control over cytokine gene expression is a key element in the regulation of the immune response. One of the transcription factor proteins that play a role in this regulation includes nuclear factor-kappaB (NF-kappaB) which is necessary for the innate immune response as well as T-cell differentiation, which is the cellular basis of acquired immunity. Environmental control over NF-kappaB transcriptional, post-transcriptional (mRNA processing and siRNA), and post-translational modifications is integral to both immune systems. This global control over cytokine expression and release as conferred by the NF-kappaB transcription factor has been termed the "enhanceosome." Epigenetic phenomena including stress play a large role in the immune-associated control over cellular differentiation including that occurring in the mammalian brain [18].

Covalent epigenetic modifications have a profound influence over gene expression and the mechanism for this effect requires environmental input and readily available substrates including target DNA and associated histones plus the methylating agent. During fetal development, it has been shown that obese pregnant mothers can pass on a pro-obese phenotype to their offspring. It has been known for some time that maternal metabolism during gestation has an imprinting effect on fetal gene expression. Indeed, this epigenetic regulation controls the divergent expression of paternal over maternal genes including one involved in glucose metabolism and growth, the insulin-like-growth factor 2 (IGF2) [19]. This fetal imprinting is the result of maternal metabolism which is indirectly linked to maternal diet. This epigenetic effect is presumed advantageous during a specific stage of gestation. However, if these epigenetic modifications sustain into later stages of gestation, they may run the risk of being a component of "maintenance methylation" which persists after parturition and into infancy, childhood, and even adult development. This may predispose the individual to new environmental pressures leading to chronic diseases such as obesity, metabolic syndrome, and cardiovascular and renal dysfunction. There is no reason to avoid speculation on these mechanisms and other disease states such as GAD.

Besides the gestational effects of maternal obesity on subsequent metabolic dysfunction in the offspring, nutritional deficiencies or excesses can also specifically alter the epigenome. Dietary sources of methylating agents such as bioavailable folic acid, methionine, choline, betaine, and homocysteine may have a permanent effect on the epigenetic methylation patterns of CpG islands and cohering histones in locus and temporal-dependent genes [20]. If these gene products are involved in normal development and have been arbitrarily altered, the fetus may not develop correctly or there may be infant diseases linked to these methylation patterns. As the individual matures to adulthood, the maternal exposure to methyl-group-containing nutrients may have a life-long effect on basic physiology, response to nutrition, and, sometimes, pathological and disease states [20].

The mechanism of chromatin methylation involves a group of enzymes. Some function directly on cytosine residues in double-stranded chromatin DNA. These DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) establish specific signatures or patterns and one of the isoforms, DNMT1, maintains the methylation and preferentially recognizes hemi-methylated DNA as substrate, thereby establishing second strand complementary methylation and a germline transference of the pattern after DNA replication [20]. It has been demonstrated that hyperinsulinemia coupled with hyperglycemia, two common outcomes in metabolic syndrome will increase the activity of DNMT and cause a general increase in hepatic DNA methylation [21]. This enhancement of DNA methylation is a direct consequence of increased flux through the homocysteine ➔ methionine ➔ adoMet pathway via an increase in both homocysteine methylation and the adoMet synthase [16].

As mentioned above, the predisposition to chronic diseases such as obesity and metabolic syndrome may arise in utero via the fetal programming mechanism. A study that examined intrauterine growth retardation in rats showed that a homeobox gene (Pdx1) became hypermethylated and therefore silenced and this led to offspring with diabetes type II disease [22]. Pdx1 is a transcriptional regulator gene involved in pancreatic beta cell functioning. In utero methylation of this gene was linked to the activity of histone deacetylase activity (HDAC 1), suggesting cross-talk in the epigenomic programming. Indeed, an increase in HDAC1 activity caused deacetylation of key histones that subsequently became methylated along with the proximal promoter region of Pdx1. This methylation pattern was conferred to the offspring and Pdx1 was completely silenced in these diabetes type II rats, suggesting that control over both DNA methylation and histone code in utero could be the direct cause of beta cell dysfunction leading to hyperinsulinaemia in the offspring [22]. It should be noted that growth retardation, as used in this study, would limit the level of growth hormone (GH) to the developing fetus. Growth hormone is secreted by the pituitary upon stimulation by the starvation hormone acyl ghrelin. Hyperinsulinaemia and gestational diabetes have been linked to childhood anxiety.

In a recent report, glucose metabolism as controlled by PDX-1-linked insulin sensitivity was linked to anxiety in an animal model [23]. This diaeventomic combination of genomic X environmental X neuroimmunoepigenomic modulation provides the molecular mechanism linking numerous childhood diseases with stress, pain, and anxiety including a strong correlate to basic human needs such as nutrition and pain avoidance.

Clearly, diet plays a major role in both general and specific epigenomic patterns and these can cause significant metabolic diseases.

Diet, both at the caloric and nutrient levels, controls metabolic flux in a dynamic way. Excess caloric intake can induce several disease states including obesity and the metabolic syndrome. Insufficient caloric intake can also cause disease as can inappropriate nutrition or excessive digestion of vitamins and certain growth-promoting molecules. Besides a direct effect on metabolic rate and function, diet can also introduce these epigenetic changes to the endocrine hormone system.

Genetic mutations that are acquired somatically can be passed on to subsequent generations and these are most commonly in the form of single nucleotide polymorphisms (SNPs) or in some, perhaps, rarer instances, copy number variations (CNVs). Once these genetic mutations have occurred and DNA has gone through a round of replication, they remain more or less fixed in the genome and become correlated with anxiety [24].

The most common cause of these genetic mutations is some kind of physical or chemical damage to the DNA as can be acquired by inflammation or environmental toxins including radiation from the sun in the form of unprotected UV light.

The epigenetic mutations on the other hand can arise within a single generation and remain fixed there, or in the mechanism of maintenance methylation, they can also be preserved and inherited. Therefore, the distinction between the two forms of modification lies more in the degree of alteration in gene expression than in the mechanisms of acquisition or potential for inheritance. Indeed, environment plays a very significant role in epigenesis of the endocrine system and may be the more robust factor in variations around this physiological axis [16]. Since the endocrine hormones play such a major role in anxiety disorders, the connection to methylation is robust.

As mentioned above, the degree of DNA or histone methylation is somewhat dependent upon the availability of biological methylating agents at the site of reaction. Therefore, adoMET and folic acid levels can be linked to the titration of these covalent modifications. The key to understand epigenetic changes is the degree of such events during critical periods of development and in particular during disease states that impact metabolism through endocrine control. Another key feature of epigenetic changes vs. genetic is the reversibility of the former and the stability of the latter. Epigenetic changes that include DNA methylation, histone acetylation, methylation, and the expression of small interfering RNA have the largest impact on protein levels and can therefore exert more profound control over metabolism than genetic modifications which may or may not effect protein expression or function [16].

It is conceivable that the mammalian uterus has an active licensing program that is involved in selective killing of certain cell masses, for example, the decidua. This would allow for preferential embryo adherence and implantation so that the initial phases of the mammalian gestation can proceed. As it turns out, sirtuin-mediated control over the interferon pathway may coordinate this licensing. The literature on this subject directs toward creating viral-free zones all the way through placental genesis that is mediated by this epigenetic reprograming (licensing) of NK cells [25]. But, maybe this is not for antiviral

purposes. In fact, interferon can be stimulated by cytosolic chromatin DNA in the form of mini-chromosomes that are the result of chromatin interrogation during times of epigenetic reprogramming [26]. This (now) cytosolic DNA triggers the interferon gamma pathway which in turn is precipitated and processed by nuclear exposure of endogenous retrovirus expression and DNA accumulation into satellite DNA endosomes via sirtuin-mediated changes in acetylation [26].

The tolerance of the fetus occurs during human gestation and could be linked to in utero epigenetic mechanisms that ultimately result in the potential for GAD in the adolescent or adult. Clearly, a diaeventomic mechanism is at play in the neuroimmunoepigenome, and imprinting of neuropsychological traits might have origins in utero.

This epigenetic reformation and realignment of the developmental program as directed by stress requires not only differential changes in multiple gene axes within cell masses embedded in complex tissues and organs including circulating leucocytes but also the bioenergetic requirements of such cells to mediate proliferation and exposure to noxious environments [27, 28].

It has been established that the switch from carbohydrate to fatty acid as the major biofuel is linked with central and peripheral inflammation. Both glycolysis and fatty acid beta-oxidation work in tandem to provide reducing equivalents for the electron transport chain and the proton pumping mitochondrial ATPase [27, 28].

Such early life adversities (ELFs) as maternal stress during pregnancy have been correlated to both anxiety disorders and the potential for the experience of pain throughout life. A recent paper has linked animal model carbon dioxide exposure and subsequent hypersensitivity to ELF-associated anxiety [29]. In this study, mice were cross-fostered in an atmosphere enriched to 6% CO_2 in the presence or absence of an acid channel ion sensing 1 gene (ACIS1) blockade drug called amiloride. When the drug was nebulized vs. administered via injection, a decrease in anxiety disorder behavior was observed. Previous work had shown an epigenetic modification of the ACIS1 expression via CO_2 enrichment that was correlated with animal distress. Indeed super-elevated concentrations of atmospheric CO_2 tend to generate a powerful pain and anxiety state in model animals and in human subjects. The link to ACIS1 expression alteration due to epigenetic modification of mRNA levels, in response to elevated CO_2, suggests this increase in gene expression is a protective response. This is a combined chemoreceptive/nociceptive stress-induced molecular modification that has been observed in the medulla oblongata where the protection centers around the response to CO_2-induced cerebral acidosis that leads to ACIS1-protective modification in respiration and nociception. Where epigenetically predisposed GAD and panic disorders are correlated with elevated CO_2 levels as induced in utero or as the result of ELF-associated choking or suffocation, the blockade of the ACIS1 gene with amiloride may be considered as a potential pharmacotherapeutic intervention [29]. The key point derived from this study is that environmental stress induces a neuroepigenomic response that may instantiate pCO_2 levels and reconditioning toward psychiatric conditions (e.g., GAD) in adults. In a study involving over 170 college-aged subjects that were genotyped according to 11 potential endogenous biomarker polymorphisms, the respiratory hypersensitivity to elevated CO_2

levels was statistically correlated to the ASIC1 common gene variant (rs1108923). This heritable variant thus segregated with a general anxiety and panic disorder-linked respiratory endophenotype [30]. Whether this gene variant would be either sufficient or necessary for respiratory distress-linked anxiety was not addressed. Upon consideration of the pathophysiological response and potential for genetic and epigenetic diaeventological interactions, it is at best a correlation that requires careful experimentation before validation of the argument.

Traumatic brain injury (TBI) is a tremendous health issue worldwide and is responsible for a considerable amount of brain-associated permanent disability and death. While physical blunt force trauma is a major source of TBI, CO_2 intoxication is also a contributing factor. Hyperbaric partial pressure of CO_2 in the blood (paCO2) alters the autoregulation of blood flow to the brain [31]. Blood vessels in the CNS respond to O_2, CO_2, and pH, and mediate mean arterial pressure (MAP) within a short window (50–150 mmHg). When MAP falls above or below this range, the capacity for autoregulation collapses and either hypotensive ischemia or hypertensive edema can result [31].

These conditions can occur in utero and throughout the post gestational life. This effectively induces neuroimmune activation that can be modified via epigenetic mechanisms leading in some instances to a potential for predisposition to GAD and other neuropsychiatric disorders. Clearly a case for a diaeventological mediated pathophysiological state.

In the specific case of $paCO_2$ effects on autoregulation, decreases cause vasoconstriction while an increase in this parameter is associated with vasodilation of the cerebral blood vessels [31]. This response is acutely sensitive to $paCO_2$ because CO_2 dissolves far better than O_2 in aqueous. However, the $paCO_2$ effect is directed to control the paO_2 for cerebral oxygen demand which is essential to prevent brain damage and death. O_2 consumption in the brain is linked to neuronal and microglial activity. As neuronal action potentials fire, this increases biological demand for O_2 to drive ATP production via metabolism and the electron transport chain/oxidative phosphorylation (ETC/OXPHOS) [31]. Both catecholamines and excitatory amino acids will increase O_2 demand and if blood flow is restricted due to the $paCO_2$, the relative concentration of neurotransmitters increases, thus potentiating neuronal damage and microglial activation to generate pro-inflammatory cytokines. When $paCO_2$ is increased, another problem arises, and this involves the increase in intracranial pressure because of excessive blood flow. This is similar to stroke associated with edema that results in the extravasation of vessel contents into surrounding tissues, thus causing an increase in interstitial fluid which will induce an immune response [31].

Increases or decreases in CO_2 levels in the brain can result in neuroimmune activation that can lead to HPA axis stimulation and ultimately the endophenotypes linked to anxiety disorders. When this occurs chronically during gestation, the fetus may obtain epigenetic alterations in key metabolic, hormonal, and immune pathways leading to a predisposition to anxiety disorders in adulthood. Likewise, this can be repeated during early infant and childhood development, and into adulthood.

5. The diaeventological axis

Living systems interact according to a three-dimensional biological trigonal plane according to the square of opposition:

A. Universal affirmative: No harm to host(s); maximum benefit to both; rare or occasional dependence.

E. Universal negation: Severe harm to hosts; benefit to only 1 host; 100% dependence.

I. Particular affirmative: benefit is disinterested; 50% dependence.

O. Particular negation: Some harm to neither; benefit to neither; no dependence for either.

This interplay involves the macrocosm, but it also appropriately describes the microcosm (human body and overall stress) imposed by the microbiome, invading pathogens, autoimmunity, cancer, autophagy, and senescence. This is the mechanism by which neuropathology is established in the CNS as described for example in glioblastoma [32].

Development, differentiation, and the signal transduction cascade network, including neuronal action potentials, neuroimmune mechanisms, and endocrine mediation, compose an opposing three-dimensional trigonal plane where the central element is the homeostasis of the existing individual.

Indeed, learning and the accumulation of memories and knowledge are all part of a massive internal interactome that can be understood compared to advantage, vectorial control, and constant failure and compensation. This is the basis for diaeventology as introduced in the introduction of this chapter.

There is a natural-native system that encompasses all of these features: the immune system.

Thus, the immune system has two roles in the human body. One is for defense and the other, in conjunction with epigenetic mechanisms, generates the existing individual with an ongoing neural network that can learn, via attention and ascent to stress on the system. This is accomplished via homologous recombination of variable regions of both the immunoglobulin family and the T-cell receptor in concert with chromatin remodeling [33], the histone code, and both the acetylome and methylome of cohering DNA [34].

If there is a link between the double aspect of mind and the body, at least one component is physical. This connection might be the molecular and cellular adaptive immunological interactome that serves to generate neural tracts according to developmental, endocrine, and peripheral stimuli, while maintaining repair processes in the CNS, by using the complex interactions between microglia and neurons.

It is well established that neuroinflammatory mediators play a critical role in the pathophysiology of brain ischemia, exerting either deleterious effects on the progression of tissue damage or beneficial roles during recovery and repair [35].

The immune response could function to generate the networked synaptic connections in the brain during development and throughout life. Soon after an ischemic insult, increased levels of cytokines and chemokines enhance the expression of adhesion molecules on cerebral endothelial cells. This causes the adhesion and transendothelial migration of circulating neutrophils and monocytes [36]. These immune cells may accumulate in the capillaries, decreasing cerebral blood flow. They can further extravasate into the brain parenchyma, thus impacting neuropsychiatric states [37].

Besides this, the infiltrating leukocytes, as well as resident brain cells, (neurons and macrophage-like microglia) may release pro-inflammatory agents like cytokines, chemokines, and oxygen/nitrogen radicals that result in tissue damage [38]. Moreover, recent studies have highlighted the involvement of matrix metalloproteinases in the propagation and regulation of neuroinflammatory responses to ischemic brain injury. These enzymes cleave protein components of the extracellular matrix such as collagen, proteoglycan, and laminin, but also process a number of cell-surface and soluble proteins, including receptors and cytokines such as interleukin-1β, thus promoting CNS inflammation and the potential for anxiety disorders [39].

The innate immune cells, macrophages, are classified into inflammatory or anti-inflammatory. Inflammatory macrophages differentiate in response to microbial and tumor antigens and interferon γ by producing pro-inflammatory cytokines at the site of nascent infection and cancerous lesions [40].

Anti-inflammatory macrophages differentiate via signaling by glucocorticoids or anti-inflammatory (type II) cytokines like IL-4, IL-13, and IL-10 where they promote TH2 immunity and mediate tissue remodeling, wound healing, and immune modulation.IL-4 and IL-13 drive anti-inflammatory macrophage polarization through the IL-4 receptor alpha chain (IL-4Rα), and anti-inflammatory polarization is also promoted by activation of several master regulators, including signal transducer and activator of transcription 6 (STAT6), Krüppel-like factor 4 (KLF4), and interferon regulatory factor 4 (IRF4), thus implicating all of these proteins in control over the generation of anxiety [40].

Macrophage polarity and activation are linked to neuropsychiatric conditions including GAD and MDD. A case in point is acute respiratory distress syndrome (ARDS) [41].

Acute respiratory distress syndrome (ARDS) is associated with an imbalance in the level of respiratory oxygen intake and CO_2 release and thus is linked to known potential pathophysiological states and GAD. ARDS can be fatal if not treated appropriately. In adults, it is associated with stiffness of the respiratory system evident in the pulmonary oxygenation step. This results in both chronic and acute hypoxemia. ARDS pathology is characterized by injury to the capillary endothelia and subsequent damage to alveoli, severe arterial vasoconstriction, and pulmonary hypertension [42]. Subsequent to lung injury, ARDS patients are typically treated with granulocyte/macrophage colony stimulating factor (GM-CSF) as a component of their pharmacotherapy [41].

GM-CSF is a pro-inflammatory cytokine associated with enhancing the level of circulating leucocytes while decreasing fetal hemoglobin levels in sickle cell anemia patients, making it a potential target for blockade with pharmacological agents [43].GM-CSF is linked to decreased oxygenation in the blood and increased immune activity-associated inflammation. While GM-CSF was also linked to depression and anxiety score elevation in ARDS patients, corticosteroids, which reduce the inflammatory response, had the opposite effect on these disorders [41].GM-CSF expression is stimulated by pro-inflammatory cytokines and inhibited by anti-inflammatory cytokines and also serves to both stimulate and regulate pro-inflammatory cytokines, thus suggesting a direct role in the activation of type I macrophages, which have been linked to GAD and associated depressive disorders [44].

Bioenergetic reprogramming is associated with macrophage polarization. The inflammatory macrophage cell type is fueled by aerobic glycolysis and can be triggered by LPS ± IFN-γ. With the anti-inflammatory lineage, IL-4 induces the expression of PPARγ, which in turn transcriptionally activates the urea cycle enzyme arginase 1 (Arg1), and the β-oxidation of fatty acids (Beta-OX) along with ETC/OXPHOS increased capacity via mitochondrial biogenesis. To fuel the anti-inflammatory bioenergetics, IL-4 also induces expression of CD36 which acts as a membrane receptor for circulating low-density lipoprotein (LDL) and VLDL-rich TAG. Finally, the unloading of TAG and associated fatty acid hydrolase activity is linked to Beta-OX, thus completing the anti-inflammatory polarization [40]. Inhibiting neuroinflammation has become a new strategy in biological psychiatry.

A recent report examines the use of a potential probiotic bacterium, *Mycobacterium vaccae* NCTC 11659 [45]. *M. vaccae* could become a biological means to treat anxiety disorders and its mechanism may involve the enhancement of T regulatory cells (T_{reg}) which act to curtail T-effector (T_{eff}) cell-meditated inflammation via the stimulation of anti-inflammatory cytokines such as IL-10, and TGF-β regionally in the hippocampus. Whether biofuel switching plays a role in this response has not been fully addressed, but elsewhere it has been reported that T_{reg}-cell metabolism toward Beta-OX and lipid utilization enhances the T_{reg} control over T_{eff}-cell-mediated inflammatory responses and further that T_{eff} cells tend to use aerobic glycolysis over Beta-OX [46].

Besides neuronal firing, hormonal signaling, cell transduction cascades, cytokine and chemokine synthesis and release, and immune cell epigenetic patterning, this biofuel connection to anxiety disorders helps to explain how in utero dietary fluctuations can affect the developing fetus.

6. Conclusions

The pre-programming of the neuroimmuno epigenome may be one of the pillars of longitudinal psychiatric disease development. A diaeventological paradigm is thus developing to explain the biological patterning of anxiety disorders. **Figure 2** below provides a developmental time course for the molecular and cellular GAD patterning described in the text.

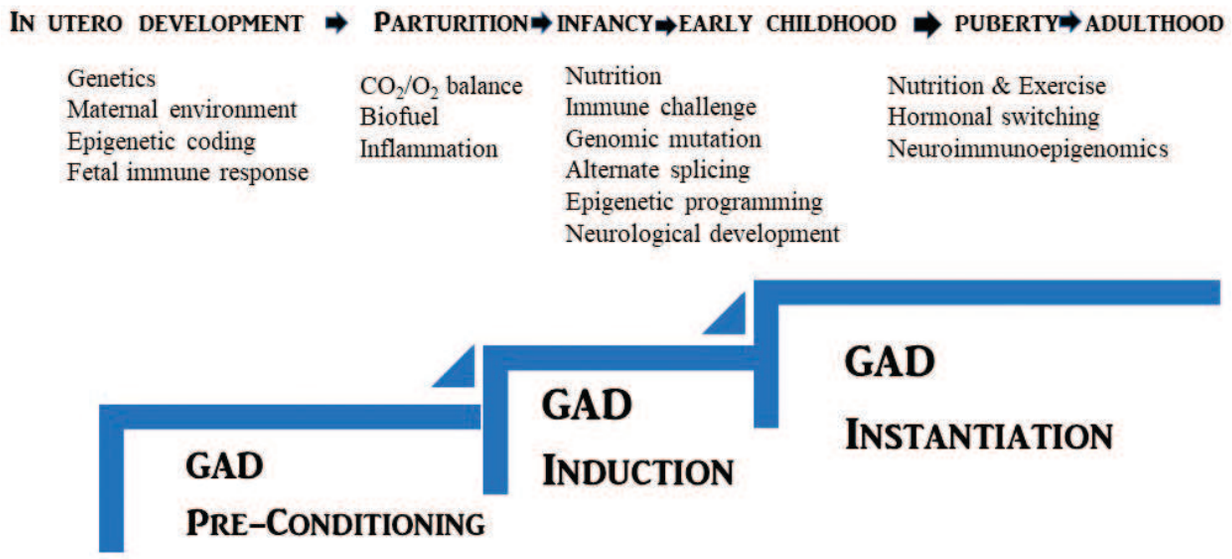

Figure 2. The diaeventomic development of anxiety disorders.

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Conflict of interest

The author claims no conflict of Interest.

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