DISCOVERING BRAIN MECHANISMS AND THE RULES OF MOLECULAR BIOLOGY

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ABSTRACT
The human genome has approximately 30,000 genes. Brain cells express at least 15,000. The human brain is subject to many acute and chronic diseases including viral encephalitis, AIDS/HIV Associated Dementia, Alzheimer's disease, and Huntington's disease. Each disease involves temporal and spatial brain gene expression and many genes have been identified involved in these diseases over several decades of research. However, with the advent of the new large-scale laboratory specimen gene expression (Microarrays) and information processing (Bioinformatics) methodologies, our data acquisition and analysis are greatly accelerated.

We need to understand the mechanisms of gene expression in the diseased brain compared to the normal brain in order to begin to develop proper molecular models for brain disease processes and thus ultimately curing these diseases. In order to carry out a project involving the human brain crucial aspects involve the tissue itself, the RNA produced the choice of many types of controls, and then the Bioinformatics procedures used. The clinical information that comes with tissue is crucial because only then can accurate disease states enter into the data analysis.

Keywords: Bioinformatics, Gene expression, Brain, Neurological Disease, Molecular Biology.

INTRODUCTION AND PURPOSE
Our understanding of the mechanisms and molecular biology of brain diseases depends critically on our ability to create information from data and knowledge from information. New laboratory techniques allow us to collect vast arrays of data. These results must be subject to well-focused analysis and must be given physiological interpretation. This requires a multi-disciplinary approach and is needed for development of logical therapies.

CYBERNETICS
We are in the Age of the Brain, Bio-enlightenment, Bioinformatics, and post-human genome. Research efforts produce data from Nanoscale level to Behavior and from basic Biology to Medical Sciences. Today as we get closer to a deep understanding of molecular processes, human behavior, social organization, and disorganization are far from understood. Norbert Wiener [64] says, "The mechanical brain does not secrete thought as the liver does bile, as the earlier materialists claimed, nor does it put it out in the form of energy, as the muscle puts out its activity. Information is information, not matter or energy. No materialism which does not admit this can survive at the present day". When Wiener, Shannon, Von Neumann, Bush, and Turing, to name a crucial few, worked on early control, communication, and computer theory, they drew on biology. We may extend this to ask whether biology obeys the laws of information.

The unifying concept created by Wiener in 1947 behind control and communication theory was Cybernetics, derived from the Greek, χυβερνιτισ steersman. He derived this new concept from information on brain and muscle (neuromuscular) function that corrective action must be taken to attain the goal or intention of an action. Thus, for example, it is a difficult process to maintain a steady gait for someone with ataxia who has nerve damage in the cerebellum. Today, we may note that this concept is also illustrated by the feed-back-loop, a concept demonstrated extensively in molecular biology. For example, a steady state may be maintained for an intermediate produced by an enzyme in a biochemical pathway due to feedback inhibition of that enzyme by one of the products in a subsequent step in the pathway. In another example, the expression of an enzyme may be induced or repressed due to need or superfluousness. The induction or repression of enzyme production may be caused by an intermediate in the pathways in which it functions.

We re-apply Wiener's ideas in the study of human brain disease. We thus return for an examination of the brain. Several decades of research have produced the notion that inflammation is one of the most damaging processes in the brain. We will discuss various types of insults to the brain including viral invasion, pre-programmed diseases, and chronic chemical abuse. However, first, we must discuss the human genome, the basis for our analysis.

THE HUMAN GENOME
The human genome has approximately 30,000 genes [34, 61], at least 15,000 of which may be expressed in any brain cell. Development of new methods is in progress to produce the global information analysis that is required to handle this information [32, 33]. These genes have a specific and finite chromosomal organization that is changeable. Fetal development, maintenance of each cell in the adult and invasion of the tissue by microorganisms each impact on the brain's defense, the immune system. Even when cells in the brain undergo a normal process of apoptosis (programmed cell death), brain cells such as astrocytes and other cells in the immune system clear the debris. This is especially apparent in late stages of fetal development when there is a normal reduction in the number of neurons [12]. Brain gene expression changes define the molecular response central in tissue health maintenance, damage, and appropriate response. In brain disease, however, genes expressed that normally have a beneficent effect, inflict damage on the brain [43].

DRUG ABUSE AND GENE EXPRESSION

Drugs of abuse have profound effects on human physiology [7, 9, 22, 23, 31]. These effects are mediated at the gene expression level and are specifically associated with neuronal malfunction [44, 45, 48, 49]. The primary drugs of abuse include cocaine and opiates that have dire social consequences i.e. associated with anti-social behaviors [18, 19, 35, 36, 37]. In addition, tetrahydrocannabinols, ethanol, and nicotine to name a few have deleterious health affects that impact severely on cost to society [19]. Cocaine and opiate drug abuse are rampant in epidemic proportions worldwide [26, 40]. Furthermore, this epidemic interacts with and fuels the AIDS epidemic [19]. Drugs and HIV have interactive effects on cells in the nervous system as well as in cell culture [17, 38, 39, 58]. Thus it is relevant for human brain disease to examine gene expression in the context of drug abuse [54].

HUMAN NEUROLOGICAL DISEASE

The human brain is subject to many acute and chronic diseases. Acute diseases include the viral encephalitides and chronic diseases include AIDS/HIV Associated Dementia, Alzheimer's disease, Multiple Sclerosis, Amyolateral Sclerosis (Lou Gehrig's disease), and Huntington's disease.

The acute viral diseases

Encephalitides in humans are cause by several viruses that infect the brain [30]. These include HIV, herpes (HSV), Varicella-Zoster (VZV), Epstein Barr (EBV), rabies, polio, measles, and St. Louis Encephalitis (SLE) viruses. Some viruses such as HIV are spread through personal contact and others such as SLE are transmitted via mosquitoes from avian species as an annual seasonal reservoir [29]. Gene expression studies of AIDS dementia have commenced [54]. As described previously, signaling molecules appear to be most important in these preliminary studies.

Chronic brain diseases have different causes.

Several chronic brain diseases are due to viral invasion and include AIDS dementia, HSV, Varicella-zoster, sub-acute Sclerosing panencephalitis (SSPE, caused by measles virus), and progressive multifocal leukoencephalitis (PML, caused by a human papovavirus termed JC [Jamestown Canyon] virus). In addition, and not to be confused with JC virus is Jakob-Creudzfeldt Disease, a brain disease like Kuru and mad cow disease that is caused by unconventional agents, prions [55, 56, 63].

Genetic cause

Huntington's disease is known to be due to an amino acid triplet-repeat expansions that occur in the Huntington gene [65] and may be associated with triplets in a few other genes as well. Triplet-repeat expansions are also implicated in the anticipation (increased risk for disease from one generation to the next) that occurs for Schizophrenia as well as Huntington's disease.

Genetic and unknown environmental factors.

Alzheimer's disease is caused by genetic (familial) abnormalities in the amyloid and presenilin genes in about 1% of cases and is sporadic in the remainder. The mechanism by which disease occurs appears to be similar for both types of disease. However, the culprits that initiate the sporadic Alzheimer's disease process are not yet identified nor understood as yet. Furthermore, as is the case with most severe brain diseases, there is no known cure.

Each disease involves (temporal and spatial) brain gene expression and many genes have been identified involved in these diseases over several decades [54]. We produced data that supported the biochemical heterogeneity of the human brain and showed that HIV evolves in the human brain with heterogeneity singular to each region of brain analyzed (basal ganglia, and temporal and frontal lobes) [50]. This is consistent with specificity and difference in the tissue biochemistry that HIV traverses as it replicates in brain. Similarly, behavioral studies related to brain structure support this approach [25, 27]. In addition, although attempts occur to repair peripheral nerve damage in AIDS at each of three levels (sural, tibial, and sciatic), repair is often heterogeneous, incomplete, or ineffective at each level [10].

RULES FOR MOLECULAR STUDIES IN HUMAN BRAIN DISEASE

In order to carry out a project involving the human brain crucial criteria must be met involving the tissue itself, the RNA produced, and the choice of many types of controls. The clinical information that comes with tissue is crucial because only then can accurate disease states enter into the data analysis [42, 50, 54, 56]. Brain tissue must be acquired in an optimum methodology. It is triaged from the Medical examiner or autopsy suite and post-mortem interval autolysis time must be minimized prior to its reaching the laboratory. This has the best possibility to provide intact RNA [53, 57, 67, 68]. The tissue must be harvested in a clean environment, to minimize possible contamination from one site to another in the brain or in the autopsy suite. These criteria for valid analysis and the methods that are required have been previously described and discussed in detail [45, 49].

An additional component of obtaining valid RNA samples for Gene expression analysis relates to the question of the actual source of material at the microscopic level. The brain is a heterogeneous organ and no two areas are alike biochemically as mentioned above [50]. Furthermore, the process of neurological disease further increases heterogeneity. Changes in cell profiles occur at the microscopic level such as increased cell numbers due to cell division (e.g. astrocystosis in PML or AIDS encephalitis) or cell infiltration (e.g. Macrophage/microglia in MS or AIDS dementia) as well as loss of cells due to cell death (e.g. apoptosis of neurons in AIDS dementia) [42]. Therefore, if RNA is analysed for small tissue aliquots, the cell heterogeneity will vitiate any attempt at uniformity and reproducibility. Thus, the use of Laser Capture Microdissection/Microscopy (LCM) is at the fore and has become crucial for gene expression studies. LCM is used to micro-dissect specific cell types that have similar staining and histological appearance. Each microdissected cell group is pooled prior to RNA purification, thus sustaining more homogeneous sample analysis (Shapshak P, Sierra-Montes J, Torres-Munos J, and Petito CK, Gene Expression Analysis in HIV Dementia and Trisomy Diseases *work in progress*, 2002).

FORMULATING BRAIN MECHANISMS

We examine a few of many approaches to analyzing and understanding the information produced in gene expression

studies and provide an outline for a basic approach. We use programs by Affymetrix, Inc. (www.affymetrix.com) and Spotfire, Inc. (www.spotfire.com) to identify and analyze changes in gene expression from oligonucleotide Microarrays. Distance, self-organizing map, and hierarchical clustering methods [24, 60] are used to determine groups of genes expression profiles that are related within the parameters defined by these means of analysis. Expressed Sequence Tags (EST's) are identified by further analysis of databases including those provided by Affymetrix and also GenBank (www.ncbi.nlm.nih.gov). Several analytic methods provide additional information for categorization. Sequence similarity search (e.g. Blast [3]) and alignment (e.g. Clustal [28]) programs are used to classify specific sequences of interest prior to distance and clustering calculations. Phylogenetic hypotheses are assessed using several programs [20, 59]. Secondary structure analysis is performed for RNA [69, 70] and protein [4; www.blocks.fhcrc.org]. Real time reverse transcriptase polymerase chain reaction (PCR) analysis is used to confirm changes in gene expression (Shapshak P and Haliko S, quantification of Presenilin-1 using real time PCR, *work in progress*, 2002; Shapshak P and Moleon-Borodowsky I, quantification of HIV using real time PCR, *work in progress*, 2002). Sequence expression in the tissue itself is confirmed in individual cells using *in situ* hybridization and immunohistochemistry separately and combined [45, 48, 51, 53, 57].

Pathways

Pathways may be derived from gene analysis studies. Pathways of anabolism and catabolism are well known; however, the new gene expression analyses produce large numbers of genes that show coordinate changes many of which have not been categorized into pathways. These potential pathways may interact in series, in parallel, and also may show overlaps. Overlaps are defined as segments of pathways that are in common in several pathways as well as shared intermediates among several pathways (e.g. branching). Convergence is exemplified by different signaling motifs binding sites on a complex promoter such as the human immunodeficiency virus (HIV) long terminal repeat (LTR) that are located on other promoters as well [8, 14, 15].

The different types of pathway structures and arrangements referred to above increase the complexity of interactions of information flow. This adds to the complexity of the gene expression function in the cell. Thus, there may be many possible arrangement of gene expression. Thus, for example, the binomial coefficient describing taking 10 items in combination from only 20 objects is $\binom{20}{10} = 184{,}756$. Thus, it is apparent that a large number of pathways are possible in the brain, even if only a small fraction of pathways may be the actually functional pathways. This type of informational approach has utility in our attempt to make sense out of the extraordinary complexity of the human genome and its pathways of gene expression. Thus, for example, there are hundreds of kinases and phosphatases. Yet, generally specific enzymes are involved in particular pathways that do not lead to confusion or inappropriate action among the many cognate enzymes. Clearly all cellular functions interact sufficiently to produce a living cell. Many of these systems within the cell may overlap providing fail-safe mechanisms and back-up against various types of sub-system failures. Thus, cytokines and chemokines communicate among the cells of the immune system as well as between brain cells and the immune system. Multiple cytokines and chemokines have overlapping and seeming degenerate functions [52]. Possible interpretations include that there are functions yet to be discovered as well as that evolution ensured the cell has mechanisms to attain a particular goal in inflammation even if one sub-system fails or is inhibited. This then becomes a major problem when maintenance of health by medical intervention requires inhibition of a particular inflammatory process for which there are overlapping sub-systems in operation in the brain. This is apparent in AIDS dementia brain with encephalitis (inflammation) where cytokines including IL-1beta, IL-6, TNF-alpha, and chemokines including MCP-1, RANTES, MIP-1 alpha, and MIP-1 beta have overlapping inflammatory effects. The induction of these damaging inflammatory molecules as well as additional compounds occurs within the same and in different cells [25, 45, 49, 46, 47, 48, 51, 66, 67]. Encephalitis in AIDS is an escalating process and it spreads in brain tissue resulting in death. It is generally unchecked since there are as yet no effective therapies.

Gene Regulatory Networks

The evolution of brain disease is understood to be a *stochastic dynamic process*, one that involves not only mutational events in genes, but epigenetic events, signaling and regulation along key pathways, production of proteins, and cellular interactions. It is likely that a range of models ultimately produce similar effect [41, 62]. It has been suggested that gene regulation can be characterized as a "scale-free" complex network, a sort of "hub-and-spoke" arrangement characterized by nodes of differing importance [1, 6]. Such networks are remarkably insensitive to error (i.e., they are *robust* to loss of nodes on average) but quite vulnerable to attack on their dominant nodes; hence, they are *fragile*.

More work must be done to understand the dynamic processes of brain disease, which can be subsumed within *gene regulatory networks*. There are several levels of analysis for features of brain disease phenotype. Clinical features are diagnosed including Schizophrenia, Alzheimer's disease, Huntington's disease, HIV Associated Dementia, etc. Pathological features are described including inflammation, apoptosis, necrosis, etc. Cell types include neurons, macrophage/monocytes, oligodendrocytes, astrocytes, macrophage/microglia, etc. We have previously made several categories of brain gene expression based on function [54]. These include N-methyl aspartic acid (NMDA) and gamma-butyric acid (GABA) neuronal receptors, Cytokines, Chemokines, Neurotransmitters, (and their respective receptors), Nitric oxide synthase, Synaptic proteins, Growth factors, maturation, Adhesion molecules/ligands, Apoptosis, Mitochondrial proteins and enzymes (non-apoptotic), Cellular toxic/inflammatory factors/ antitoxins, DNA binding proteins, Signal transduction pathways, Transcription and RNA binding, Cell cycle regulation, Cytoskeleton regulation and collagen, Microtubule/filament and microtubule/filament binding, and Pseudogenes, to list several [54]. These characteristics can be viewed as potential output properties of the gene regulatory network [2, 5, 11, 13, 16, 21]. Gene

regulatory networks express effects on the time-rate-of-change (or *derivative*) of variables, such as

$$\frac{d\mathrm{x}}{dt} = \dot{\mathrm{x}} = \mathrm{f}[\mathrm{x}, \mathrm{u}, \mathrm{w}, \mathrm{p}, \mathrm{h}(\mathrm{x}, \mathrm{u}, \mathrm{w}), t]$$

where **x** is the dynamic state of the system (a vector of features that are regulated by genes, including genes themselves, proteins, and characteristics of cells), **u** contains exogenous controlled inputs (e.g., therapeutic treatment), **w** contains environmental disturbances and other exogenous effects, **p** is a vector of network parameters, **h**(**x**, **u**, **w**) is a neural network whose argument may contain the state or exogenous causal factors, and *t* is time. The integral solution to this coupled, nonlinear, multivariate equation is a history of **x** from start to end, portraying evolution of the disease over time.

Gene regulatory networks provide the mathematical framework for studying genetic and epigenetic effects on brain disease development. The mathematics provide an additional dimension for understanding progression of the disease, affording a powerful basis for studying optimized policies of prevention and treatment.

CONCLUSIONS

As mentioned above, early control, communication, and computer theory drew on biology for ideas and paradigms. We now reverse the process and ask whether biology obeys the laws of information theory. We do not have an answer yet but our approach and hypothesis are that biology obeys these laws.

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