

# Chips for Brains

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**Abstract:** Large scale analysis of gene expression using DNA arrays shows great promise in enhancing our understanding of how the genome constructs the brain. This technology has been employed to analyze gene expression variations in the brain resulting from genetic polymorphisms, drug abuse, aging, and the neuropsychiatric disorders such as schizophrenia and Alzheimer's disease. Although the data from DNA array analysis is necessarily noisy, thoughtfully designed experiments can give useful insights. In addition, as the use of DNA array technology grows, replicated experiments will provide even greater confidence in drawn conclusions, giving optimism that these methods will yield profound insights into the genomics of the brain.

## INTRODUCTION

An enduring mystery is how the linear sequence of instructions in the genome gives rise to the staggering complexity of the brain, and how this program goes awry in disease. The prospects of obtaining genomic views of neural gene expression changes seemed remote until the advent of new approaches using multiplex methods. These technologies, referred to as DNA arrays, microarrays or gene chips, have vastly improved the throughput of gene expression analysis and are revolutionizing our understanding of how gene networks respond to various genetic, environmental and other disturbances. There are at present two widely employed approaches for creating DNA arrays. The first of these employs high speed robotic printing of transcript sequences, for example cDNAs, onto glass slides [1]. An alternate approach employs photolithography and spatially addressable combinatorial chemical synthesis in order to build high-density arrays of oligonucleotides on glass [2]. Alternative approaches include ink jet technology, either for placement of presynthesized DNA or *in situ* oligonucleotide synthesis on glass, and fiber optic bundles, in which each fiber contains an oligonucleotide at its terminus [3].

The major issue confronting DNA array studies of the brain is related to the extremely noisy nature of the data. This is due to a number of factors, including only fair sensitivity of the DNA array technology, uncertainties due to lack of absolute quantitation, poor comparability between studies, and high variability in sample values, exacerbated by the heterogeneity and cellular complexity of the brain. A number of important experimental and mathematical tools are being developed to distill the best possible information

from DNA array studies, and the area remains one of active research [4-9]. Despite the current shortcomings of the technology, there are solid grounds for hope that with increasing sophistication of experimental and analytic tools, valuable insights can be obtained into the genetic molecular workings of the brain using DNA arrays. A number of intriguing studies have recently been published which bolster this viewpoint, and give fresh insights into the relationship between the genome and the brain.

## COMPARISON OF BRAIN REGIONS FROM DIFFERENT INBRED MOUSE STRAINS

The relative contribution of genes to interindividual variation is a source of continuing fascination, but there are a number of obstacles to directly studying this phenomenon in humans. Most prominent amongst these difficulties are minimization of environmental variations and uncontrolled genetic variability. The mouse offers distinct advantages in these regards, and presents the possibility of probing genetic causes of variation through the study of different inbred strains under controlled conditions. In a recent pioneering study, gene expression variations between the inbred mouse strains C57BL/6 and 129SvEv, were investigated using oligonucleotide arrays with 10,000 genes [10-12]. These two inbred strains display a number of behavioral differences. Gene expression was analyzed in the amygdala, cerebellum, cortex, entorhinal cortex, hippocampus and midbrain. It was found that 24 genes were significantly differentially expressed between the two strains in all six brain regions, and a total of 73 genes were differentially expressed between strains in at least one brain region. Surprisingly, however, within strains, only 70 genes showed clear differences between cortex, cerebellum and midbrain. This unexpected finding suggests that variation in brain gene expression is more marked between strains than between brain regions. The overall findings of this study suggest that DNA array technology may be a useful tool to identify genes responsible for inter-strain variations.

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## PROFILING PSYCHOSIS GENES

It is clear that schizophrenia has a strong genetic component, but it has been very difficult to identify the responsible genes, probably because of the myriad difficulties that confront human genetic analysis of complex traits. These obstacles include genetic heterogeneity, environmental contributions and the frequently marked polygenic nature of psychiatric disorders [13, 14]. Two interesting studies have recently appeared that employed DNA arrays to identify potential genes that may play a role in schizophrenia. The first study employed microarrays to look at dorsal prefrontal cortex in schizophrenic brains compared with control subjects [15]. This brain region was studied as it had been implicated in the causation of the disorder, both from cellular and behavioral viewpoints. A matched sample design was employed, in which each microarray was co-hybridized with RNA from a schizophrenic brain and a matched normal control. The RNA samples were differentially labeled using Cy3 or Cy5. The rationale behind the use of matched samples was increased sensitivity of the analysis, which helped circumvent the errors from gene expression changes due to systematic effects such as aging. This thoughtful design will therefore detect even modest gene expression changes. Despite the use of this safeguard, *post-hoc* analysis suggested that even pooled samples would still have identified a number of significantly regulated genes.

Hierarchical data analysis was employed on the matched sample dataset, in which genes were grouped into 250 functionally related clusters. The analysis revealed that genes involved in regulating presynaptic function were decreased in all subjects with schizophrenia. Importantly, selected genes in this group were carefully verified using *in situ* hybridization. Two of the most consistently changed genes in the presynaptic group were NSF (N-ethylmaleimide sensitive factor) and SYN2 (synapsin II). Interestingly, these two genes were replicated in an additional set of samples. The observed gene expression differences were not due to treatment with antipsychotic medication, since the changes were also found in subjects who were not receiving drug therapy at the time of death. To further ensure that the observed gene expression differences were relevant to schizophrenia, the frontal cortex of haloperidol-treated and control monkeys was analyzed using microarrays and *in situ* hybridization. No changes in the presynaptic gene group were found in this experiment, increasing confidence in the potential importance of these genes to the schizophrenia disease process.

The second paper [16] also looked at the dorsolateral prefrontal cortex. Oligonucleotide gene chips were used for this study, and a model-based meta-analysis approach employed for interpretation of the data. This investigation found dysregulation in schizophrenia of genes involved in a diverse range of biological processes, including neuronal development, signal transduction, neurotransmission and synaptic plasticity. One of the most interesting findings was the marked down regulation in schizophrenic brains of genes involved in myelination, suggesting a role for oligoden-

drocyte function in the disorder. Based on the expression profiles of the significantly differentially expressed genes, linear discriminant analysis was employed to assess the differences between the normal and schizophrenic samples. Interestingly, this approach was able to separate the diseased and normal groups and showed increased power when restricted to myelination related genes. Further analysis revealed no differences in the conclusions when the data was stratified into antipsychotic treated and non-treated groups, suggesting that the findings were not artifacts due to drug therapy.

The discrepancies between the two studies discussed here are most likely due to differences in patient populations, as well as in the genes on the arrays. It will be interesting to see if a consensus emerges from further studies of larger population groups. In the meantime, it will be worthwhile to compare the results from these DNA array studies with those from potential mouse models of schizophrenia, both genetic and pharmacological [17-21].

Another study sought candidate genes potentially involved in mania and psychosis [22]. The prefrontal cortex and amygdala of rats treated with methamphetamine as a model of mania were analyzed for gene expression changes using oligonucleotide arrays. Genes significantly regulated as a result of this procedure were searched against genomic loci implicated in either bipolar disorder or schizophrenia, on the basis of human genetic mapping studies. Genes were considered to be candidates if they showed significant expression changes in the rat pharmacologic model and also mapped to within 10 cM of a genetic marker with suggestive evidence of linkage. These criteria were met by eight genes, which included G protein-coupled receptor kinase 3, D-box binding protein, farnesyl-diphosphate farnesyltransferase 1, vertebrate LIN7 homolog 1, sulfotransferase 1 and insulin-like growth factor 1.

## SEEKING GENES FOR SLEEP

The genetic control of the sleep-wake cycle is an almost completely unexplored and mysterious area. Both differential display and cDNA microarrays were employed to systematically explore the gene expression changes that occur in the cerebral cortex of rats during sleep and waking, giving a combined total survey of about 10,000 genes [23]. A tally of 44 genes were found that were induced in the waking state relative to sleeping, and 10 in the sleeping state relative to waking. The known genes upregulated in waking could be grouped into immediate early genes, chaperones, synapse related genes, neurotransmitter receptors, hormone receptors, neurotransmitter transporters, and enzymes, as well as a miscellaneous group. The remaining genes upregulated in waking, and all those upregulated in sleep, with one exception, were novel genes. The study included a broad-ranging confirmation of the identified genes using *in situ* hybridization, RNase protection and real-time quantitative RT-PCR. It will be interesting to see if any of the identified sleep/wake genes are also regulated in mouse [24] and dog [25] models of narcolepsy.

## ANALYZING ALZHEIMER'S DISEASE

The ability to analyze gene expression profiles from single cells was a dramatic advance, which occurred through the development of antisense RNA (aRNA) amplification technology [26]. This methodology has been used in conjunction with cDNA microarrays to compare expression profiles of neurofibrillary tangle-bearing and normal CA1 neurons from sections of Alzheimer's and normal brains, respectively [27]. The amplified aRNA gave impressive sensitivity, allowing the quantification of transcripts from single neurons that had been immunohistochemically identified and recovered from fixed post-mortem human brain sections. Regulation of selected genes was confirmed by replication using reverse Northern blot analysis to custom cDNA arrays. It was found that neurons with neurofibrillary tangles in Alzheimer's brains displayed significant decreases in expression levels of a number of classes of genes previously implicated in the disease. These classes of genes included signal transduction molecules, cytoskeletal proteins, synaptic proteins and neurotransmitter receptors for glutamate and dopamine. In addition, regulated genes were found that had not been previously thought to be involved in Alzheimer's disease. These genes included focal adhesion kinase, glutaredoxin and utrophin, and may possibly play novel roles in neurofibrillary tangle formation or degeneration.

## GENES INVOLVED IN DRUG ABUSE

Drug addiction is a major health problem, with devastating social consequences [28, 29]. A number of individual genes have been implicated in drug addiction on the basis of expression studies, and the relevant signaling mechanisms include those in cAMP and opioid receptor pathways, as well as in brain stress systems that feature corticotrophin-releasing factor (CRF). However, despite these interesting insights, there has been so far a paucity of studies to examine gene expression changes due to drug addiction at a global level.

In an interesting recent investigation [30], the effects on rat hippocampus of the primary psychoactive component of marijuana,  $\Delta^9$ -Tetrahydrocannabinol ( $\Delta^9$ -THC), were analyzed. The study looked at changes due to both acute and chronic exposure to  $\Delta^9$ -THC. Verification for selected genes employed RNA dot-blot analysis as well as *in situ* hybridization. A total of 49 genes were significantly altered by  $\Delta^9$ -THC exposure, of which 28 were known, 10 had homologies to ESTs and 11 displayed no known homologies. Cannabinoid administration was found to alter expression of a number of genes, including prostaglandin D synthase and calmodulin, both of which are thought to be involved in endogenous cannabinoid synthesis or effector systems. The induction of prostaglandin D synthase by cannabinoids is likely related to arachidonic acid release, while regulation of the calmodulin gene may reflect the phasic changes in cAMP levels which occur as a result of tolerance to cannabis. In addition, other genes, which had a less clear relationship to cannabinoid function were found to be regulated by  $\Delta^9$ -THC, including neural cell adhesion molecule (NCAM) and myelin basic protein (MBP).

## UNDERSTANDING ALCOHOLISM

Alcoholism also results in a substantial socioeconomic burden, but the molecular changes that result in the brain from this disorder are poorly understood. One recent study analyzed postmortem specimens of superior frontal cortex of alcoholics and non-alcoholic controls [31]. This region was chosen because of its importance in decision-making, judgement and other executive functions, as well as its selective vulnerability to the effects of chronic alcohol abuse. Pooled samples of RNA were employed from specimens consisting of two independent groups of controls and two of alcoholics. The pooled sample strategy was employed to reduce case-to-case differences unrelated to alcoholism. The samples were then analyzed using both cDNA and oligonucleotide arrays. Most emphasis in the analysis was placed on genes with similar results in both case groups and on both types of array. Out of 4,000 genes assessed, 163 genes were found to significantly differ between alcoholics and non-alcoholics. Interestingly, the most prominently affected genes were those involved in myelin function, which were down-regulated in the alcoholic cortex. This may be related to the well known reduction of the cerebral white matter volume in chronic alcoholism. In addition, genes involved in cell-cycle, as well as other known neuronal genes were found to be altered in their expression levels.

Another study employed oligonucleotide arrays to investigate the gene expression changes due to chronic alcohol exposure in a neuroblastoma cell line [32]. Regulation of selected genes by alcohol was confirmed by Northern blots, Western blots and ELISA. After three days of treatment with ethanol, a set of 42 genes were found which had consistently increased or decreased transcript abundance. Most prominently affected were groups of genes related to norepinephrine production, glutathione metabolism and anti-apoptosis. The regulation of genes involved in catecholamine metabolism may be related to the role of this pathway in the physical dependence and withdrawal symptoms of alcoholism. Interestingly, alcohol decreased levels of monocyte chemoattractant protein 1, a chemokine with important proatherogenic roles. This observation may partly explain the protective effects of moderate alcohol consumption on heart disease. Furthermore, a subset of genes were found which were similarly regulated by both ethanol and dibutryl-cAMP. This may be related to the findings of a role for cAMP in ethanol intoxication in *Drosophila* and drinking preference in mice.

## A STIMULATING ENVIRONMENT FOR GENE EXPRESSION REGULATION

Studies have shown that rodents reared in an enriched environment display long-term improvements in performance on various tests of learning and memory. To identify the genetic pathways responsible for these alterations, oligonucleotide arrays were employed to identify cortical gene expression changes in mice exposed to enriched environments for between three hours to fourteen days [33]. The enriched environment included such objects as various toys, small birdhouses and a spin wheel, and the items were changed or rearranged every half-day. The

enriched animals were compared with littermate controls, which were maintained in standard laboratory conditions. Labeled RNA samples were hybridized twice to two different arrays, and consistent differences in the replicates were further analyzed. The arrays contained about 11,000 genes. A large number of genes were found whose expression changed in response to the enrichment training. A total of 60 genes were consistently altered by environmental enrichment at the early time points, and approximately 100 genes at the later times. The early regulated genes could be grouped most prominently into those involved in macromolecular synthesis, neuronal signaling, neuronal growth and structure, and proteases involved in cell signaling and apoptosis. Interestingly, the later regulated genes were largely distinct from the early genes, and were mostly related to neuronal transmission and structural changes.

### ANALYZING AGING

The exact genetic mechanisms responsible for the impairments in cognitive and motor skills that occur due to aging are not known. To better understand the genome-wide changes that occur in the brain as a result of this process, oligonucleotide arrays were employed to determine the patterns of gene regulation in the neocortex and cerebellum in aging mice compared to adult controls [33a]. Selected candidate genes were confirmed using real-time quantitative RT-PCR. The mice were autopsied in order to exclude those animals with overt disease. It was found that aging resulted in a gene expression profile in the brain that suggested elements of oxidative stress, an inflammatory response, and reduced neurotrophic support. Interestingly, it was found that caloric restriction, which is known to retard mammalian aging and prolong life-span, selectively inhibited the upregulation due to aging of the stress and inflammatory response genes.

### GLOBAL GENOMICS

What are likely future developments in the use of DNA arrays to study the brain? It seems clear that present DNA array technology can give reliable conclusions, provided studies are carefully and thoughtfully designed, and replication employed where necessary. However, one increasingly important issue will be how to assess the biological and behavioral significance of candidate genes uncovered through DNA array analysis. High throughput mutagenesis [34], transgenesis [35, 36] and gene knockouts [37] in model organisms will be useful, as will studies based on high throughput genotyping of humans [38]. Nevertheless, new fundamental approaches are clearly needed to drive down the cost and labor of such studies.

Another area in which some room for controversy exists is the relative role of human and animal models in DNA array studies. Humans have the advantage of disease validity, but it is nearly impossible to provide precisely matched controls for these investigations. In contrast there will always be doubts concerning disease validity in mice, especially for afflictions such as the neuropsychiatric

disorders, where the etiology is uncertain. However, the mouse does provide the possibility of carefully controlled experiments. In the long run, the best insights will probably be obtained by judicious combined use of both human and animal models, which will allow for the best use of the complementary advantages and disadvantages of these organisms.

A significant consensus that is emerging is the necessity of centralized public domain databases for DNA array data. This should facilitate replication and careful evaluation of datasets, helping to increase confidence in the conclusions from DNA array studies. However, such an undertaking will involve major challenges. Even relatively conceptually simple matters, such as the most effective and flexible methods for deposition of the raw data, are open to animated and enthusiastic debate. This leaves aside more subtle and demanding issues of comparability of experimental design and sample choice. However, concerns over data sharing are not a new phenomenon. Similar debates took place in the arenas of DNA sequence analysis [39] and protein structure determination [40], and are currently taking place in brain mapping [41]. In the first two cases, public access to data emerged as the accepted consensus. Thus, despite the considerable effort required to confront the issues of establishing centralized DNA array databases, there is little doubt that the necessary investments will be repaid many fold. In fact, a database towards this end, Gene Expression Omnibus, or GEO, has recently been established at the National Center for Biotechnology Information (NCBI: <http://www.ncbi.nlm.nih.gov>). The success of such a database would lead to an unprecedented era of international and global genomic cooperation, where meta-analysis of data from many groups would become a routine and accepted part of scientific practice. The consensus and robust new insights obtained from such cooperation has great promise for revolutionizing our understanding of the brain in both health and disease.

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### ABBREVIATIONS

<sup>9</sup> -THC	=	<sup>9</sup> -Tetrahydrocannabinol
aRNA	=	Antisense RNA
CRF	=	Corticotrophin-releasing factor
GEO	=	Gene Expression Omnibus
MBP	=	Myelin basic protein
NCAM	=	Neural cell adhesion molecule

NCBI = National Center for Biotechnology Information  
 NSF = N-ethylmaleimide sensitive factor  
 SYN2 = Synapsin II

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