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# Genome Scale Mapping of Brain Gene Expression

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*Two new approaches, voxelation and gene expression tomography (GET), permit multiplex acquisition of gene expression patterns in the brain. Both methods result in volumetric images of gene expression analogous to those produced in biomedical imaging systems. Voxelation employs analysis of spatially registered cubes from the brain, whereas GET entails analysis of parallel slices obtained by rotation about multiple axes. These methods have been used to investigate neurologic diseases and their models in both humans and mice. The results of these studies are discussed, as is the future of high-throughput gene expression mapping in the brain. Biol Psychiatry 2003;53:1069–1074 © 2003 Society of Biological Psychiatry*

**Key Words:** Brain mapping, expression profiling, functional genomics, gene expression tomography, microarrays, voxelation

## Introduction

In understanding how the one-dimensional structure of the genome gives rise to the immense three-dimensional (3D) complexity of the brain, knowledge of all gene expression patterns will be of tremendous value. A number of approaches currently exist for mapping gene expression patterns. In situ hybridization and immunocytochemistry are sensitive and allow gene expression patterns to be acquired at cellular resolution. Instrumentation for automated in situ hybridization has recently been developed and is being employed for large-scale acquisition of gene expression patterns in the brain (Herzig et al 2001). Additional approaches use analysis of targeted regions of the brain obtained from laser capture microdissection or micropunches. An alternative method links gene expression patterns in the brain with overexpression phenotypes (Heintz 2001). This strategy employs high-throughput creation of transgenic mice harboring fusions of the green fluorescent protein gene with genes of interest. Another promising approach, imaging mass spectrometry, maps protein levels in the brain rather than transcript levels

(Stoeckli et al 2001); however, this methodology uses expert level instrumentation and, in addition, requires time-consuming assignment of mass spectrum peaks to individual proteins. Although there are tools available for 3D imaging of gene expression in living organisms, these methods currently only permit examination of one or a few genes at a time (Contag et al 2000; Herschman et al 2000).

Many of the existing techniques can offer excellent spatial resolution, often at the cellular level. Their major disadvantage is low throughput. For example, in the case of laser capture microdissection, there are more than 700 anatomically distinct named major regions in the mammalian brain (Franklin and Paxinos 1997; Virtual Hospital n.d.) and potentially  $> 10^5$  histologically distinguishable cell types. Furthermore, named structures, such as the cortex, have a wide variety of histologically distinguishable cytoarchitectonic domains. Thus, despite many impressive advances, it is daunting to think of using the currently available methodologies to obtain comprehensive overviews of gene expression patterns for all brain diseases of interest, both in humans and model organisms.

High-throughput array technologies for gene expression profiling have provided valuable insights in unicellular systems (Brown and Botstein 1999; Lipshutz et al 1999) but have yet to be widely employed to understand how the 3D structure of the brain is derived from its genome. In contrast, biomedical imaging technologies, such as computed tomography, positron emission tomography, and magnetic imaging resonance, provide vivid and detailed images of structure and function in the brain (Frackowiak et al 1997; Mazziotta and Toga 2002) but are low-throughput. Here, we review two new methodologies, voxelation and gene expression tomography (GET) that aim to combine many of the advantages of gene expression profiling and biomedical imaging while minimizing a number of their disadvantages to provide a high-throughput approach to mapping 3D gene expression patterns in the brain.

## Voxelation and GET

The principles underlying voxelation and GET are shown in Figure 1. Both methods allow high-throughput acquisition of multiple volumetric maps of gene expression by

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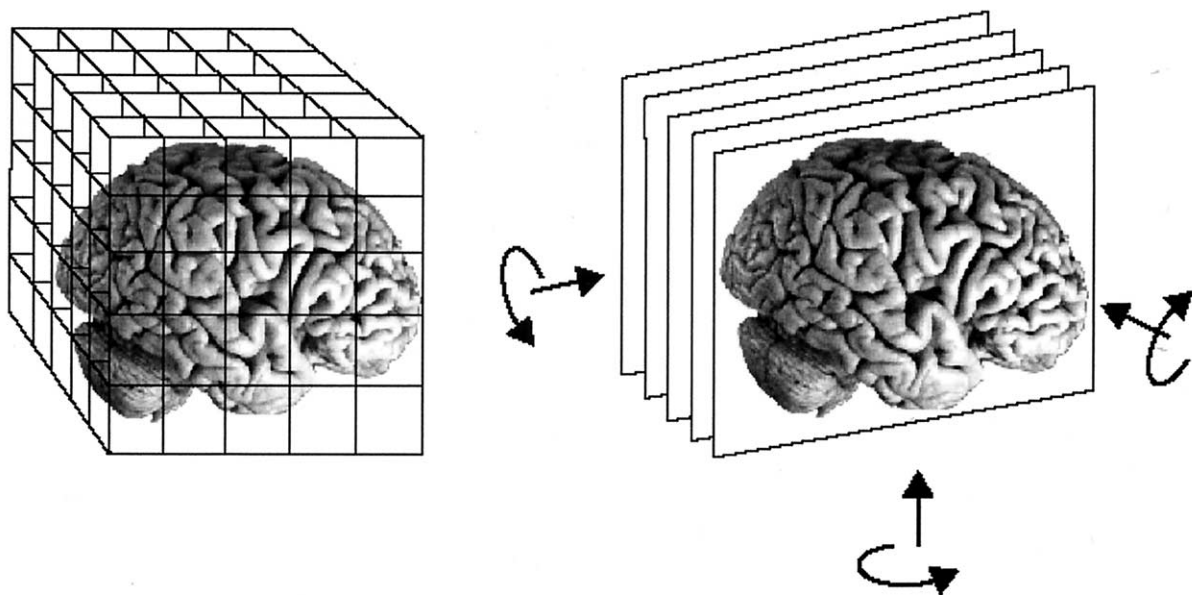


Figure 1. Principles underlying voxelation (left) and gene expression tomography (right).

reconstruction of data from parcelated specimens. The essential idea is the simplification of complex 3D anatomy into arrays of biochemical samples, providing valuable opportunities for scalability and automation.

Voxelation is named from the term “voxel,” the 3D analog of the pixel. The method employs high-throughput analysis of spatially registered voxels harvested from the brain (Figure 1), followed by 3D reconstruction (Brown et al 2002a, 2002c). In contrast, GET employs analyses of sets of parallel slices or “views” obtained from the brain by progressive rotation about multiple independent axes (Figure 1) (Brown et al 2002b). Tomographic image reconstruction can then be employed for reconstruction of gene expression patterns. Interestingly, orthogonal slices were used in the 1960s to map releasing factors in the brain by triangulation (Crighton et al 1970); however, these approaches only employed views at right angles and indeed were introduced before the advent of computerized tomography in biomedicine. The methods are thus not tomographic and lack the potential resolving power and image reconstruction capabilities of GET.

The resolution of voxelation depends on the volume of the voxels employed: the smaller the voxel, the higher the resolution. The resolution of GET also depends on voxel size. The total number of voxels that can be obtained from a GET reconstruction equals the number of samples analyzed =  $AVS$  (where  $A$  = the number of axes of rotation,  $V$  = the number of views per axes, and  $S$  = the number of slices per view). If  $V_0$  = the volume of the brain, the volumetric resolution of a GET reconstruction =  $V_0/AVS$ .

#### *Voxelation of the Human Brain*

Voxelation has been employed to analyze coronal hemisections from normal and Alzheimer’s disease brains at the level of the hippocampus (Brown et al 2002a). The hemisections were divided into 24 voxels of  $\sim 1 \text{ cm}^3$ , and each voxel was analyzed using a 2000 gene microarray. Despite the relatively crude spatial maps, it was possible to extract useful information from the resulting gene expression patterns.

One of the most salient insights that emerged was from the use of singular value decomposition (SVD) or principal component analysis. A powerful mathematical tool, SVD is commonly used in biomedical imaging that clusters the data into principal components with maximum explanatory power for the variance in the data. It is entirely data driven and does not rely on preconceived notions or hypotheses. The first four principal components emerging from the SVD analysis of the human voxelation data are shown in Figure 2. These images do not represent the expression pattern of any one gene, but rather the expression patterns of gene “vectors,” each comprising hundreds of genes, which together may be particularly important in specifying the regions in which they are expressed. Similar to what is found in biomedical imaging, the first principal component was uniformly expressed in all voxels, an average representation of the whole brain. The second principal component was largely restricted to the cortex, portraying a gene vector that may play an important role in specifying this brain region. The third principal component was expressed in the tail of

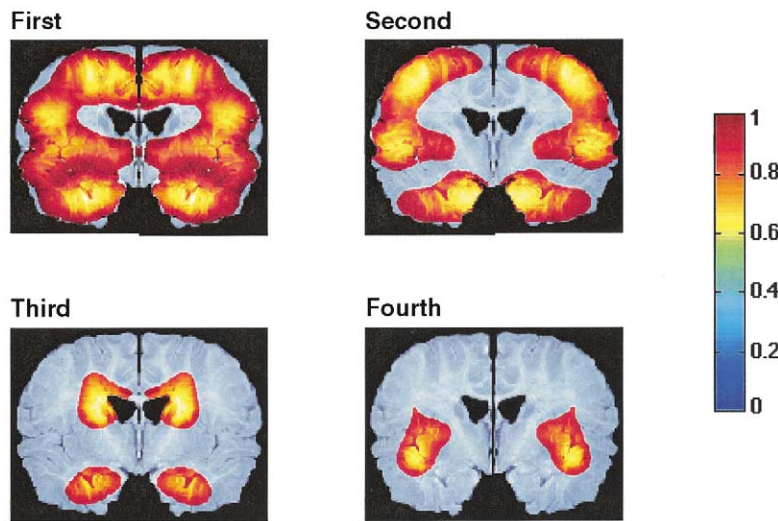


Figure 2. Singular value decomposition delineates anatomic regions of the human brain. The spatial patterns resulting from the first, second, third, and fourth principal components (gene vectors) are shown. The conjoint matrix obtained from the top 120 genes that are most strongly ( $p \sim .05$ ) differentially expressed between the normal and Alzheimer's hemisections was employed for this analysis. The level of expression of the relevant gene vector can be deduced by reference to the pseudocolor scale (far right). Imaging software smoothed the expression patterns over the voxels, and the hemisection was reflected along the midline giving bilateral symmetry.

caudate and the hippocampus, suggesting a hitherto unsuspected genetic connection between these otherwise disparate areas of the brain. Expression of the fourth principal component was restricted to the insular cortex, a region of the brain that plays an important role in the language capabilities of humans.

Other analyses were also performed using the voxelation data. Genes were found that were consistently differentially expressed between the normal and Alzheimer's disease brain. These genes were involved in a number of molecular activities including signal transduction, transcription, modulation of the cytoskeleton, and cholesterol synthesis. In addition, networks of genes with correlated expression patterns were found conserved between the normal and Alzheimer's disease samples. By seeking noncoding sequences shared between these genes, it was possible to identify putative control regions responsible for their coregulation.

#### *Voxelation of the Mouse Brain*

Voxelation has also been used to examine brains from normal mice and mice in which a model of Parkinson's disease had been induced using methamphetamine (Brown et al 2002c). The voxelation scheme divided the whole brain into 40 voxels, an average volumetric resolution of 7.5  $\mu\text{L}$ . Each of the voxels was analyzed using a 9000-gene microarray, giving 9000 gene expression patterns. Similar to the human voxelation studies, a number of analyses were performed using the mouse data. Two mutually exclusive clusters of coregulated genes were found in the mouse brain (Figure 3), one expressed anteriorly and one expressed posteriorly, in the cerebellum. Interestingly, the clusters were maintained in both the normal and Parkinson's disease brain. By seeking con-

served noncoding sequences, it was possible to identify putative regulatory regions responsible for these correlated gene expression patterns.

Genes consistently differentially expressed across all voxels of the control and Parkinson's disease brains were sought. It was found that genes involved in cell-cell interactions were most frequently regulated in the Parkinson's disease model, suggesting that neurite outgrowth may be an important response to the loss of dopaminergic neurons that occurs in this disorder. An SVD analysis demonstrated a dramatic shift of gene vectors away from the striatum in the Parkinson's disease brain. Because the striatum is known to be strongly affected in Parkinson's disease, this finding suggests it will be possible to use gene expression profiling in combination with voxelation to identify regions of functionally abnormal neuroanatomy. This approach may be particularly valuable in the neuropsychiatric disorders such as schizophrenia, autism, and Down syndrome where, despite many years of study, the functionally responsible brain regions remain unclear.

#### *Gene Expression Tomography*

Gene expression tomography has recently been used to reconstruct images of tyrosine hydroxylase (TH) gene expression in the mouse brain (Brown et al 2002b). Tyrosine hydroxylase transcript levels were quantitated in the harvested slices using real-time polymerase chain reaction (PCR) or RNase protection, and gene expression images reconstructed using filtered back projection (Figure 4). Within the expected limits of spatial resolution, the reconstructed TH gene expression patterns were highly similar to the known pattern (Min et al 1994; Stork et al 1994), with the strongest expression being found in the midbrain (substantia nigra and ventral tegmental area),

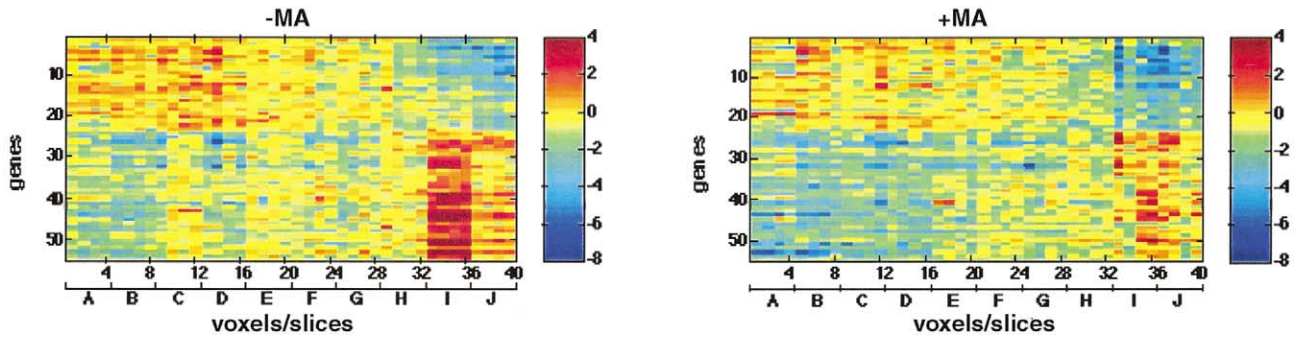


Figure 3. Spatial gene expression patterns for a subset of genes in the control (–MA) and methamphetamine (+MA)-treated mouse brains. Two mutually exclusive clusters of coregulated genes were found, conserved between the control and experimental brains. One cluster is expressed anteriorly and the other posteriorly, in the cerebellum, suggesting that these clusters play a role in the distinction between anterior and posterior. The relative level of expression of any gene in any voxel is read by looking along the relevant row and column, finding the intersection, and referring to the scales. The numbering of the columns corresponds to the voxels (1–40), and the lettering (A–J) corresponds to the slices. The voxel numbering is such that anterior-most voxels are represented by the left-most columns, and the posterior-most voxels are represented by right-most columns.

ventral diencephalon (periventricular and paraventricular hypothalamic nuclei, zona incerta), and pons (locus coeruleus), areas in which TH transcripts are expressed at high levels. No known regions of expression of the TH gene were absent in the GET reconstructions.

The nominal volumetric resolution of the GET reconstruction was 3  $\mu$ L, a linear resolution of 1.5 mm. It was expected that this resolution should allow successful separation of bilaterally symmetric parasagittal structures, such as the left and right substantia nigra and locus coeruleus. This expectation was fulfilled (Figure 4). The good quality of the images reconstructed using GET was further confirmed from a Monte Carlo analyses, which showed that these images had a statistically significant level of similarity to the known TH expression pattern.



Figure 4. Gene expression tomography image reconstruction of the tyrosine hydroxylase gene expression pattern. Bilateral expression of the gene can be seen in the locus coeruleus.

## Discussion

Two main factors limit information recovery from voxelation and GET: voxel inhomogeneity and the performance of the methods used to analyze the voxels and slices. Voxel inhomogeneity is an inevitable consequence of finite voxel size (noninfinitesimal resolution). Genes expressed in a small subset of cells within a voxel will have their signal “diluted” by adjacent nonexpressing cells, the partial volume effect. A considerable proportion of these genes will be of interest; however, inhomogeneity becomes less of a problem as voxel size decreases and hence resolution improves. For initial studies of voxelation and GET (Brown et al 2002a, 2002b, 2002c), simple approaches to registered harvesting of voxels and slices were sufficient and used available tools such as the cryostat; however, higher resolution maps will require dedicated instruments for semiautomated harvesting of miniaturized voxels and slices.

Two devices have recently been constructed for voxelation that allow recovery of 1-mm voxels from the rodent brain and 3.3-mm voxels from the human brain (Singh et al, in press). The device for the rodent brain results in a volumetric resolution of 1  $\mu$ L, equivalent to a 300 voxel map for the whole mouse brain. Construction of the device required photoetching combined with precision lamination to create blades of adequately small dimensions. This is probably close to the limits that can be achieved using solely mechanical means and smaller voxels may require alternate approaches (e.g., laser beams or microelectromechanical systems technologies). An additional strategy to minimizing voxel size is to select specific subsets of cells within voxels using a lineage-specific marker such as green fluorescent protein (Peterson 2002).

The figures of merit for the tools used to analyze voxels include throughput, dynamic range, sensitivity, and reproducibility. Microarrays have excellent throughput and reasonable dynamic range. In terms of throughput, for the mouse model of Parkinson's disease 9000 genes were examined—approximately 25%–30% of the genome. Forthcoming generations of arrays should even more fully interrogate the genome. Microarrays have limited sensitivity and reproducibility, and improvements are clearly needed here. In terms of sensitivity, array analysis can be replaced by real-time PCR for selected genes. Although this technique does not have the throughput of microarrays, it does have exquisite sensitivity. In terms of reproducibility, microarrays can reliably distinguish twofold differences in transcript abundance. It is likely that this is comparable to in situ hybridization because the foundation of the two methods, hybridization, is the same. An additional source of noise for voxelation and GET is interindividual variation. Again, this is likely to be no worse than that for in situ hybridization or any of the other available methods.

Despite improved resolution, the disadvantages of smaller voxel sizes in voxelation and GET are diminished recovery of RNA and the larger numbers of samples required to analyze the entire brain. The first factor necessitates adequate sensitivity for the transcript profiling tools and the second increases cost. Currently microarrays are sufficiently sensitive to allow construction of  $> 10^6$  voxel maps of the human brain. Assuming 30,000 genes, this would imply a data set of  $> 10^{10}$ , large indeed (Peterson 2002). In addition, to be feasible for repeated analyses, the cost per voxel for such a map would have to drop by  $\sim 10^3$  to about 10 cents. Nevertheless, it is currently realistic to consider the construction of a 2000-voxel map of the human brain, that would provide images of reasonably high quality. For creation of high-quality expression maps of the mouse brain, linear RNA amplification techniques (Kacharina et al 1999) should readily permit creation of multiplex gene expression maps of 300 voxels using the recently constructed high-resolution voxelation device described above (Singh et al, in press).

In comparing voxelation and GET, the latter has the advantage that slices as thin as 10  $\mu\text{m}$  can be readily harvested using the cryostat. In principle, therefore, gene expression images obtained using GET could reach the level of resolution of the light microscope; however, voxelation has the advantage that the voxels will have less inhomogeneity than the slices obtained using GET, and hence voxelation should have reduced partial volume effects and better image contrast. Combined use of voxelation and GET may thus be of value in certain situations by providing data sets with complementary strengths and weaknesses.

What are the advantages of voxelation and GET? Despite information losses due to voxel inhomogeneity and the limitations of the tools used to analyze samples, it is clear that because of their throughput, these new genomic imaging strategies should provide opportunities for greater net information recovery than is possible using techniques such as in situ hybridization. In particular, voxelation and GET should permit analyses of gene expression in many brain disorders and their models, difficult with the classical methods. Another advantage of voxelation and GET is their modality independence, in principle allowing mapping of the proteome, metabolome, conceivably even electrophysiology, in addition to transcript abundance. Thus, despite their relatively recent development, the genomic scale information provided by voxelation and GET is likely to help achieve the ultimate goal of understanding how the conceptual wiring diagram of the genome gives rise to the neuronal wiring diagram of the brain in both health and disease.

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