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Neuropsychopharmacology. Author manuscript; available in PMC 2009 April 30.

Published in final edited form as:
Neuropsychopharmacology. 2009 January ; 34(1): 18–54. doi:10.1038/npp.2008.172.

Target Identification for CNS Diseases by Transcriptional Profiling

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Abstract

Gene expression changes in neuropsychiatric and neurodegenerative disorders, and gene responses to therapeutic drugs, provide new ways to identify central nervous system (CNS) targets for drug discovery. This review summarizes gene and pathway targets replicated in expression profiling of human postmortem brain, animal models, and cell culture studies. Analysis of isolated human neurons implicates targets for Alzheimer's disease and the cognitive decline associated with normal aging and mild cognitive impairment. In addition to τ, amyloid-β precursor protein, and amyloid-β peptides (Aβ), these targets include all three high-affinity neurotrophin receptors and the fibroblast growth factor (FGF) system, synapse markers, glutamate receptors (GluRs) and transporters, and dopamine (DA) receptors, particularly the D2 subtype. Gene-based candidates for Parkinson's disease (PD) include the ubiquitin–proteosome system, scavengers of reactive oxygen species, brain-derived neurotrophic factor (BDNF), its receptor, TrkB, and downstream target early growth response 1, Nurr-1, and signaling through protein kinase C and RAS pathways. Increasing variability and decreases in brain mRNA production from middle age to old age suggest that cognitive impairments during normal aging may be addressed by drugs that restore antioxidant, DNA repair, and synaptic functions including those of DA to levels of younger adults. Studies in schizophrenia identify robust decreases in genes for GABA function, including glutamic acid decarboxylase, HINT1, glutamate transport and GluRs, BDNF and TrkB, numerous 14-3-3 protein family members, and decreases in genes for CNS synaptic and metabolic functions, particularly glycolysis and ATP generation. Many of these metabolic genes are increased by insulin and muscarinic agonism, both of which are therapeutic in psychosis. Differential genomic signals are relatively sparse in bipolar disorder, but include deficiencies in the expression of 14-3-3 protein members, implicating these chaperone proteins and the neurotransmitter pathways they support as possible drug targets. Brains from persons with major depressive disorder reveal decreased expression for genes in glutamate transport and metabolism, neurotrophic signaling (eg, FGF, BDNF and VGF), and MAP kinase pathways. Increases in these pathways in the brains of

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DISCLOSURE/CONFLICT OF INTEREST

Dr Marquis Vawter is a co-inventor on three patent applications regarding gene expression for several genes described in this article. If any of the pending patent applications are accepted, Dr Vawter could eventually receive a partial share of royalties. Otherwise, the authors declare that, except for income received from our primary employers, no financial support or compensation has been received from any individual or corporate entity over the past three years, and no personal financial holdings exist, that could be perceived as constituting a potential conflict of interest.

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animals exposed to electroconvulsive shock and antidepressant treatments identify neurotrophic and angiogenic growth factors and second messenger stimulation as therapeutic approaches for the treatment of depression.

Keywords

microarray; gene expression; schizophrenia; bipolar disorder; depression; Alzheimer's disease; Parkinson's disease

INTRODUCTION

Because of the slow pace of innovations in the therapy of central nervous system (CNS) diseases and a growing elderly population, disabilities due to neurodegenerative and psychiatric diseases now represent the second most frequent cause of morbidity and premature mortality in the USA. Most psychiatric drugs being approved today are refinements and reformulations of drugs discovered decades ago by serendipitous clinical observations. *Bona fide* targets for neurological disease are fewer in number, and proof-of-concept studies are rare in neurological practice.

Improved technologies for gene expression analysis of the human brain, confirmation of identified genes by RNA- and protein-based assays, and gene-based compound screening have identified new targets for CNS disease. This mechanistic approach to neuropsychiatric and neurodegenerative drug discovery is highlighted in this review, which is limited to genes and their protein products that satisfy at least one of the following criteria: (1) Did their discovery confirm or extend other messenger ribonucleic acid (mRNA)- or protein-based findings? (2) Did they identify proven pathways or targets, or those that are under strong consideration? (3) Did existing, or emerging, drugs confirm the target in proof-of-concept studies? Most of the genes described in this review that met or exceeded these criteria are summarized in Table 1 for neurodegenerative diseases, and Table 2 for psychiatric diseases, in the order that they appear in the text.

Conceptual Approaches

Traditional CNS drug discovery is a target-oriented 'top–down' approach that screens new compounds based on their interaction with an established receptor or other target. This approach may explain why most new drugs duplicate the efficacy and side effect profiles of their predecessors, and ameliorate only a portion of disease pathology. Most CNS diseases are treated with a combination of drugs, and some of the most effective drugs such as the antipsychotic clozapine (Roth *et al*, 2004; Altar *et al*, 1986, 2003a) and the mood stabilizers lithium, valproate, and carbamazepine (Gould *et al*, 2004) interact with the widest number of targets, not the fewest as one might imagine. In addition, family and association studies of DNA haplotypes show that most single gene mutations explain at best only a small portion of the variation in psychiatric, cognitive, or neurodegenerative disorders, and that better genetic association scores are obtained when multiple genes are considered (Tsuang *et al*, 1999; Pulver, 2000; Plomin and McClearn, 1993; Hauser and Pericak-Vance, 2000; Pericak-Vance *et al*, 2000). In the absence of a deeper understanding of disease pathology and mechanisms of side effects, CNS drug discovery would remain dominated by the redesign of drugs for familiar targets and reduced approval rates for 'me too' drugs.

The ability to evaluate changes in the expression of the entire genome in brain areas affected by CNS disease and drug effects on multiple pathways is an alternative, 'bottom–up' approach to drug discovery (Palfreyman *et al*, 2002; Ogden *et al*, 2004; Le-Niculescu *et al*, 2007). Most human cells contain a nucleus and, within that organelle, the same DNA. It is

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the differential expression of genomic DNA, in the form of mRNA, and its transcription into protein, that determines the type and function of each cell. Insights into disease pathology can be revealed by the 'disease signature' (Figure 1), those genes whose expression in the brains of patients differs reliably from those of non-afflicted, matched controls (Palfreyman *et al*, 2002). The treatment of cultured human neural cells or experimental animals with therapeutic compounds that target the disease can produce a 'drug signature' of genes, the expression levels of which are changed in common in response to multiple therapeutic drugs for that disease. This pharmacogenomic approach to drug discovery is a clear paradigm shift from the conventional method of re-deriving drugs with a known mode of action.

Combining Disease and Drug Signatures for Target and Drug Discovery

The discovery of genes that change in model systems in response to therapeutic agents, and in a direction that is opposite to their change in disease may provide drugable targets, and novel mechanism(s) which address a broader biochemical basis of disease (Figure 1). The statistical, biological, and pharmacological significance of each of these target genes can be calculated by an algorithm to determine which genes to use in a large-scale mRNA-based drug screening program (Table Box 1). Genes identified in these ways can be used to screen small molecules for activity. New receptor targets can be identified by the pharmacology of novel compounds identified, and, if these are agonists, by the ability of selective antagonists to block their downstream gene effects.

Genomic Tools for Understanding Disease

High-throughput mRNA technologies (Ginsberg *et al*, 2006d; Lockhart *et al*, 1996; Schena *et al*, 1995; Brown and Botstein, 1999), including microarray platforms, are at the center of this paradigm shift. They provide an affordable, simultaneous assessment of gene expression levels in many samples in a single experiment. These technologies remain technically challenging, and require high-quality RNA to measure the relatively small (20–50%) changes in brain gene expression (Eberwine *et al*, 2001; Ginsberg *et al*, 2004; Jurata *et al*, 2004). They also require validation by independent methods, including real-time quantitative PCR (qPCR) and measurements of their respective protein products. These methods, and, well-characterized postmortem human brain samples, high-throughput spotted cDNA expression miniarrays, and methods to extract RNA from single neurons and discrete cell clusters in brain, are described below.

TECHNOLOGICAL ADVANCEMENTS FOR THE FUNCTIONAL GENOMICS APPROACH

Suitable Tissue Sampling

Human brain tissue—The study of CNS gene changes in neurological or psychiatric cases requires brains collected from well-documented cases. Collections of neurodegenerative disease samples include those of the Multiple Sclerosis Brain Bank and the New South Wales Brain Bank, part of the Australian Brain Bank Network (Sheedy *et al*, 2008), the University of Miami Brain Endowment Bank, the Center for Neurodegenerative Disease Research at the University of Pennsylvania, the collection at UC Davis, the New York Brain Bank at Columbia University (Vonsattel *et al*, 2008), the Religious Orders Study at the Rush University Medical Center (Bennett *et al*, 2002; Mufson *et al*, 2007a), and the Cognitive Neurology and Alzheimer's Disease (AD) Center at Northwestern University. In Appendix A, we include a reference to a comprehensive list of brain banks focused on neurodegenerative diseases and maintained by the National Institute of Neurological Disorders and Stroke (NINDS). Prominent brain collections with an emphasis on psychiatric disease include the Stanley Medical Research Institute (SMRI) (Johnston *et al*, 1997; Torrey

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et al, 2000), the collection at UC Irvine (Vawter *et al*, 2004b; Bunney *et al*, 2003), the University of Pittsburgh (Mirnics *et al*, 2000; Mirnics and Lewis, 2001), and the Harvard Brain Tissue Resource Center, which has psychiatric and neurodegenerative disorder collections (Konradi *et al*, 2004; Benes and Berretta, 2000) (Appendix A).

Requisite clinical information on brain samples includes subtype of the CNS disorder, pathological diagnosis, therapeutic treatments, cause of death, antemortem state, concomitant medical conditions, and lifestyle information such as cognitive performance, smoking, alcohol intake, medication history, and evidence of substance abuse (Torrey *et al*, 2000; Katsel *et al*, 2005b). Other necessary measures at autopsy include postmortem interval, brain pH, patient age, sex, and brain weight. Unfortunately, even the best-documented collections contain gaps in such information, such as the amount and frequency of therapeutic and other drug treatments, cognitive status, and state of the illness at the time of death. Other limitations of brain banks can include the dearth of age-matched control cases, particularly in AD, Parkinson's disease (PD), and schizophrenia research, between-center differences in anatomical dissection methods, or the scarcity of frozen sections, the analysis of which by laser capture microdissection (LCM) can mitigate against variations in dissection methods. The prospective diagnosis and well-planned collection of samples, as exemplified by the Religious Orders Study of the Rush University Medical Center (Mufson *et al*, 2003), is advantageous in contrast to retrospective disease diagnosis. These advantages include the collection of more accurate patient demographics, finer delineation of patient subtypes, shorter postmortem intervals, and other variables that can be co-analyzed with the results of microarray studies.

In addition to the importance of RNA quality to gene expression profiles, tissue pH appears to correlate highly with the expression of particular gene classes, including those of metabolic and hydrogen ion transport functions (Johnston *et al*, 1997; Li *et al*, 2004; Altar *et al*, 2005; Vawter *et al*, 2006b; Knable *et al*, 2002; Bahn *et al*, 2001). It remains unknown whether decreases in brain pH lower the expression of these genes, or whether their decreased expression contributes to brain acidosis during the antemortem agonal period.

Several laboratories have evaluated the effects of different freezing and fixation protocols on RNA quality, ease of tissue microdissection, and success of cDNA array analysis (Bahn *et al*, 2001; Van Deerlin *et al*, 2002; Vincent *et al*, 2002). For example, the agonal state of a patient prior to death can have profound effects on several parameters, including RNA stability and protein degradation, as hypoxia, pneumonia, and protracted coma have been associated with alterations in RNA and protein levels (Li *et al*, 2004; Barton *et al*, 1993; Hynd *et al*, 2003; Tomita *et al*, 2004). These antemortem variables and postmortem tissue procedures must be considered in molecular studies of human postmortem tissues. Of critical importance is the assessment of RNA quality and quantity, particularly for discrete cell RNA assessments. Acridine orange (AO) histofluorescence provides a rapid assessment of RNA quality in tissue sections. AO is a fluorescent dye that intercalates selectively into nucleic acids, and has been used to detect RNA and DNA in brain tissues (Ginsberg *et al*, 1997, 2006d; Vincent *et al*, 2002; Mikel and Becker, 1991). RNA quality can be obtained with high sensitivity using bioanalysis (eg, 2100 Bioanalyzer; Agilent Technologies). Bioanalysis enables visualization of capillary gel electro-phoresis results in an electropherogram and/or digital gel formats. DNA and protein quality and abundance can also be evaluated by bioanalysis (Freeman and Hemby, 2004).

Microaspiration and microdissection of specific cell types—Heterogeneity of neuronal and non-neuronal cell populations characterizes the mammalian brain. Normative brain function and dysfunction, are mediated in large part through gene expression, and ultimately protein expression, within these interconnected cells. The collection of specific

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cell types from brain is challenging, but often necessary, as evidenced by the limitations of studying relatively large 'blocks' of brain tissue for gene expression experiments. These blocks contain not only the neurons of interest but also unaffected neurons and other cell types, including astrocytes, glia, and vascular tissues. The unaffected cells will most likely greatly outnumber the affected cells and obscure the disease signature. This problem can be circumvented by two recently developed methods for collecting homogeneous cell populations from thin sections.

Single-cell microaspiration entails visualizing cell(s) using an inverted microscope connected to a micromanipulator, microcontrolled vacuum source, and an imaging workstation on an air table. Individual cells can be aspirated from the tissue section and placed in microfuge tubes for RNA amplification. Microaspiration can accurately dissect neurons, neuropil, or dendrites identified by a specific immunostain with minimal disruption of the surrounding neuropil (Ginsberg *et al*, 2006d; Hemby *et al*, 2002, 2003; Crino *et al*, 1998; Ginsberg and Mirnics, 2006). This is demonstrated for a $p75^{NTR}$-immunoreactive cholinergic neuron in the nucleus basalis of an AD patient (Figure 2). The mRNA of single or pooled cells can be measured by qPCR, or amplified for subsequent microarray analysis.

LCM enriches the detection of cell-specific genes from populations of cells (Ginsberg *et al*, 2006d; Jurata *et al*, 2004; Altar *et al*, 2005; Luo *et al*, 1999) (Figure 3). LCM employs a high-energy laser source that separates desired cells from the remaining tissue section, and facilitates transfer of the identified cells for analysis (Bonner *et al*, 1997; Emmert-Buck *et al*, 1996). Positive extraction LCM, as exemplified by the PixCell IIe system from Arcturus (MDS Analytical Technologies, Sunnyvale, CA), directs a 'laser beam' onto the cells of interest for the purpose of microaspiration (Figure 3). Negative extraction LCM, or non-contact laser extraction, employs a laser source to cut around the area of interest within a tissue section, and the circumscribed material is catapulted into a microfuge tube. This method is utilized by the PALM system (PALM Microlaser Technologies, Bernried, Germany) (Ginsberg *et al*, 2006d). Both positive and negative extraction methods allow captured cells and their processes to be examined microscopically to confirm the identity and quality of isolated cell population(s). Single cells as well as dozens to hundreds of cells can be collected by LCM instrumentation. RNA, DNA, and protein extraction methods can be performed on microdissected cells (Fend *et al*, 1999; Goldsworthy *et al*, 1999; Simone *et al*, 2000), although LCM is used primarily for RNA extraction and subsequent cDNA microarray analysis. Positive extraction methods have an advantage of allowing for relatively quick and consistent microaspiration of desired cells. Negative extraction can be more time consuming, but can precisely control the cells or areas in a tissue section that are microdissected and catapulted.

RNA Detection and Verification

cDNA- and oligonucleotide-based gene microarrays are slightly different technologies for quantifying mRNA for each gene represented on the array (Jurata *et al*, 2004). Commercially available microarrays contain probes for 15 000–30 000 different mRNA species, and allow gene expression from much of the human genome to be measured in each sample in the course of several days of experiments.

cDNA arrays—cDNA arrays have been widely used to profile human brain tissue in the study of psychiatric and neurodegenerative disorders (Ginsberg *et al*, 2004, 2006b, c; Mirnics *et al*, 2000; Altar *et al*, 2005; Middleton *et al*, 2002; Vawter *et al*, 2001; Ginsberg, 2007; Colangelo *et al*, 2002; Lukiw, 2004). cDNA arrays are constructed with picoliter spots of double-stranded PCR products amplified from cDNA libraries or by plasmid preparation of individual cDNAs and/or expressed sequence tagged (EST) cDNAs. Double-stranded

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cDNAs/ESTs are printed in a tiny grid pattern on specially prepared glass slides or nylon membranes. The labeling of a control and a disease sample RNA with either of two resolvable fluorescent labeling dyes, such as cyanine 3 and cyanine 5, allows one to simultaneously measure relative gene expression levels in two samples on the same cDNA array. However, the haphazard pairing of control and disease samples and dye bias effects have generated spurious results. Though twice as costly, it is more reliable and statistically more powerful to pair each sample with a common reference derived from similar tissue, such as a pool of the control group samples (Jurata *et al*, 2004; Altar *et al*, 2005; Konig *et al*, 2004). The pairing of each sample with the same internal reference cDNA minimizes false positives due to local background, dye bias effects, or printing inconsistencies between arrays (Jurata *et al*, 2004).

cDNA arrays are washed to remove nonspecific back-ground hybridization, and a laser scanner images biotiny-lated/fluorescently labeled probes, whereas a phosphor imager is used for radioactively labeled probes. The specific signal intensity (minus background) of RNA bound to each probe is expressed as a ratio of that signal to the total hybridization signal intensity of the array. This minimizes variations across the array platform due to differences in the specific activity of the probe and the absolute quantity of probe present (Ginsberg *et al*, 2006d; Ginsberg and Mirnics, 2006). An additional advantage of cDNA microarray technology includes the full representation of 3′ ends of genes, which is especially important for detecting the expression of low levels of RNA that require linear amplification prior to microarray analysis (Altar *et al*, 2005).

Oligonucleotide arrays—Oligonucleotide probes are deposited by a photolithographic printing process that lessens differences between arrays (Lockhart *et al*, 1996). Oligo arrays have been used by investigators in psychiatric brain research for their extensive coverage of the genome, relatively easy sample processing, and generally reliable data (Prabakaran *et al*, 2004; Hakak *et al*, 2001). Each labeled sample is hybridized to its own array for a direct comparison to be made between intensity data of individual samples. As an example, each gene on the Affymetrix GeneChip® (Santa Clara, CA) is represented by eleven 25-mer oligonucleotides that are spaced throughout the length of the transcript, and nearly identical oligonucleotides containing a single mismatched base provide information about nonspecific hybridization. Disadvantages of oligonucleotide arrays include decreased sensitivity for detecting samples with a 3′ bias (ie, generated by linear amplification, or predominate in poor quality mRNA), requirement for a relatively large input amount of starting RNA, and their lack of an internal hybridization reference to reduce the effects of local artifacts (Jurata *et al*, 2004).

Advancements in microarray technology—In addition to RNA that is transcribed from DNA and translated into a protein, the transcriptome includes transcripts that are not translated into protein. These non-coding RNA transcripts include ribosomal RNA (rRNA), transfer RNA (tRNA), short hairpin RNA, short interfering RNA, small nuclear RNA transcripts, microRNAs (miRNAs) and other antisense transcripts with regulatory functions. Fortunately, newer microarray technologies encompass this greater transcriptome breadth with innovations such as the exon array, mRNA array, and ChIP array. These advanced microarray platforms use 20–60 oligonucleotide base pair probes, whereas earlier cDNA platforms generally contained 300–600 base pair probes. The shorter oligonucleotides can measure a greater number, and more specific, transcript regions and splice variants of each gene. This improves the sensitivity and specificity of microarrays, and reveals between-individual variations in gene transcription. This is important, as about 40% of the human transcriptome is composed of alternatively spliced genes. As illustrated for glutathione S-transferase M1 (Figure 4), these variations can be measured between individuals. It is likely that a greater understanding of CNS disorders will result from SNP analysis, and other high-

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throughput evaluations of RNA editing, DNA methylation, transcription factor immuno-precipitation, promoter binding assays, and diversity of transcripts and proteins. Commercial and non-commercial microarray platforms and software sources to conduct microarray gene expression studies are listed in Appendix B.

Processing array data—Microarray data can be processed by calculating the average ratio between control and disease groups, or untreated and treated samples, and analyzed by ANOVA to determine whether the changes are statistically significant. Corrections for multiple testing such as Bonferroni (multiplying a *p*-value by the number of comparisons), or the less stringent Benjamini and Hochberg false discovery rate method, can help reduce the number of false positives that are invariably obtained with microarray experiments (Kaminski and Friedman, 2002; Yang *et al*, 2002; Reiner *et al*, 2003).

One common approach is to cluster samples according to their common patterns of differences in gene expression. This can produce the familiar heat map color-coded illustrations that might shed light on clinical sub-populations of disease. More commonly, however, sample clustering reflects confounding variables such as patient drug history or poor RNA preparations. Such confounds can produce equally impressive 'clustering' within just the control group, or within two groups created at random from the pool of all control and patient samples. The absence of such clustering within the control group or mixed groups would increase confidence in the clustering of genes in the control *vs* experimental group comparison. Gene clustering can define functional relationships among the altered genes when they vary in a similar manner across most or all of the samples of a treatment or disease group *vs* the controls (Kaminski and Friedman, 2002).

Pathway analysis tests a list of candidate genes by the Fisher's exact test or hypergeometric distribution for over-representation of genes in that pathway relative to the entire set of genes on a microarray platform. The results of such pathway tests are corrected for multiple comparisons to establish the false discovery threshold. Another useful method to identify biological pathways that are implicated by virtue of changes in a large number of their gene members is the Expression Analysis Systematic Explorer (EASE; http://david.abcc.ncifcrf.gov/ease/ease.jsp) (Hosack *et al*, 2003). EASE can determine whether there is a statistical over-representation of genes involved in a particular biochemical pathway or cellular class defined by Gene Ontology (http://www.geneontology.org/), when compared to the representation of that pathway or class on the array (Altar *et al*, 2005, 2008). EASE can also be applied to proteins from a proteomic platform, or other high-content methods. Pathway analyses by EASE, Ingenuity, ermineJ, GeneGO, and other methods are indispensable tools to identify changes in functional pathways from groups of genes with nominal significance and fold changes.

RNA verification—Regardless of how statistically impressive microarray data may appear, it is essential to verify at least a subset of gene expression changes by an independent method. cDNA microarrays can contain large percentages of improperly annotated probes (Kuo *et al*, 2002; Kothapalli *et al*, 2002), which can create false-positive gene hits. The nonspecific hybridization or cross-hybridization of closely related genes to oligo or cDNA probes can also yield false positives.

Real-time qPCR—Amplification of genetic signals can be performed at both DNA and RNA levels, and final amplified products are either DNA or RNA. A common method to measure DNA or RNA is the (PCR (Mullis, 1990) and is invaluable in confirming gene changes in postmortem human brain RNA (Johnston *et al*, 1997). Starting material for PCR reactions can originate from genomic DNA or cDNA reverse transcribed from RNA (eg, RT-PCR). PCR is an effective method to amplify a DNA template. However, PCR is an

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exponential, nonlinear amplification, and variation can occur within individual mRNA species of different molecular mass or base pair composition. PCR-based methods tend to amplify abundant genes over rare genes and may distort quantitative relationships among gene populations (Phillips and Eberwine, 1996). Real-time qPCR can quantitate PCR product formation during each cycle of amplification and generates fewer concerns than those associated with conventional PCR methods. Other advantages of real-time qPCR include higher throughput, the ability to simultaneously multiplex reactions, greater sensitivity, reduced inter-assay variation, and lack of post-PCR manipulations (Bustin, 2002). Fluorescence is generated by labeled nucleotides that are incorporated into the gene-specific PCR product, or a labeled internal probe emits fluorescence on its displacement by the generation of the PCR product. The enhancement of fluorescent signal is monitored at multiple times in each cycle of PCR, thus the name ‘real time’ is used to distinguish the process from assays that are based on end point analysis.

Real-time qPCR can generate false negatives, primer–dimer pairings, variability in cDNA synthesis, and cross-reactivity with genomic DNA. These issues can be surmounted by targeting primers near the gene region of interest, incorporating melting curve dissociation analysis for primer–dimer detection, use of DNAse treatments to rid the sample of contamination, resequencing amplicons and, when possible, design of primers spanning two exons.

The comparison of data from different qPCR studies requires normalization of qPCR data sets to control for signal fluctuations due to the stepwise qPCR process and sample variability. A normalizing approach can include the use of housekeeping gene(s), total RNA input, or the number of cells used. Selecting an appropriate reference standard for normalization is crucial, as variation in the standard can produce equally significant errors in mRNA quantification as in between-study variations. One common approach is to generate standard curves and cycle threshold (C_t) values using standards obtained from total brain RNA. The $\Delta\Delta C_t$ is often employed to determine relative gene-level differences normalized by the qPCR products of housekeeping genes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-actin, and cyclophillin as a control (Ginsberg and Mirnics, 2006; ABI, 2004). Alternatively, NormFInder and geNorm are algorithms that identify genes for normalization by ranking genes in the experiment according to their stability of expression (Ohl *et al*, 2005; Andersen *et al*, 2004).

High-Throughput mRNA Screening

The identification of reciprocal gene expression changes in disease and drug signatures has allowed the validation of gene responses to other drugs using high-throughput methods. One of these, termed a multi-parameter highthroughput screen^{SM} (MPHTS^{SM}), incorporates a miniarray of 16 relatively large hybridization spots (Figure 5) deposited on the bottom of each well in a 96-well format (High Throughput Genomics, Tucson, AZ) (Martel *et al*, 2002). This platform measures transcript abundance through an RNase protection step, coupled to the hybridization of the protected fragment that is between a specific homing oligo printed in the bottom of each well, and a universal oligo reporter linked to horseradish peroxidase. Detection is accomplished through a chemiluminescent reaction and photon capturing by a CCD camera (www.htgenomics.com). Advantages of MPHTS^{SM} are that it does not rely on RNA amplification, decreasing cost and signal variability. When genes of moderate to high abundance are used, MPHTS^{SM} generates intra- and interplate coefficients of variation of around 5 and 10%, respectively. These low levels of variation allow the accurate measurement of 20% or greater changes in gene expression.

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THERAPEUTIC TARGET AND DRUG DISCOVERY

Neurodegenerative Disease

Alzheimer's disease—Age is the greatest risk factor for developing mild cognitive impairment (MCI) and AD. The likelihood of developing AD doubles every 5 years after the age of 65 years, so that by the age of 85 years, the risk approaches 50%. Thus, it is highly significant that the number of USA citizens who are 65 years of age or older will double, from the 35 million alive today, by the year 2030 (Plassman *et al*, 2007). About 4.5 million Americans have AD today, and this number may reach 11–16 million by 2050 (Plassman *et al*, 2007; Wimo *et al*, 2007). The aging of this population demands greater public health efforts to combat cognitive decline and lessen future costs of caring for people with dementing illness. Although such costs far eclipse those of monetary concern, financial costs alone are estimated to exceed US$315 billion worldwide in 2005 (Wimo *et al*, 2007). It is imperative that we learn more about the causes of AD to develop rational therapies that can delay its onset or progression.

Dementia impairs the ability to learn, reason, make judgments, communicate, and carry out daily activities. The progressive decreases in cognitive and memory functions typically lead to the diagnosis of AD and, from that point, death within 7–10 years. AD accounts for approximately 50–60% of dementia cases worldwide. It is a multifactorial disorder, and is likely to originate from complex genetic and environmental risk factors. AD follows at least a bimodal distribution, whereby rare (<3%) autosomal dominant mutations cause early-onset familial forms of AD, whereas the common sporadic form of later-onset AD is determined by genetic polymorphisms with fairly low penetrance but much higher prevalence (Bertram *et al*, 2007; Swerdlow, 2007).

The neuropathology of AD is characterized by filamentous material in intracellular and extracellular compartments in the form of neurofibrillary tangles (NFTs). These contain hyperphosphorylated τ. Senile plaques (SPs) consist primarily of amyloid-β (Aβ) peptides, and both markers of AD are characteristically found in the hippocampal formation and temporal neocortex (Mufson *et al*, 2003; Ginsberg *et al*, 1999b; Selkoe, 1997; Trojanowski and Lee, 2005; Hyman and Trojanowski, 1997; Mirra *et al*, 1991). Among the various neurotransmitter systems that deteriorate as AD progresses, the most consistent deficits and cell losses are in long projection neurons including those of the cholinergic nucleus basalis (Mufson *et al*, 2007a, b; Whitehouse *et al*, 1982) and glutamatergic neurons within the entorhinal cortex and neocortex (Ginsberg *et al*, 1999b; Hyman *et al*, 1984). Diagnosis of AD is confirmed by the postmortem presence of amyloid plaques and NFTs in the brain of a patient who displayed progressive cognitive decline (Hyman and Trojanowski, 1997; Mirra *et al*, 1991).

A goal of translational profiling has been to identify genes, their regulatory elements, and pathways that contribute to NFT and SP deposition, and the change in the expression of which correlates with cognitive decline in AD. Microarray analysis has been an effective tool to assess transcript levels in animal models and postmortem brain of AD (Table 1) (Ginsberg *et al*, 2006a,b;Colangelo *et al*, 2002;Lukiw, 2004;Loring *et al*, 2001;Blalock *et al*, 2004;Dickey *et al*, 2003;Mirnics *et al*, 2003;Reddy *et al*, 2004;Miller *et al*, 2008). The majority of microarray studies of neurodegenerative disease have used gross brain dissections as the basis for expression profiling. Given the limitations discussed earlier about this approach, the microaspiration of neurons, NFTs, and SPs within the hippocampal formation and nucleus basalis of human postmortem tissues and animal models have proven to be an effective paradigm shift. These studies have yielded provocative data sets relevant to the pathophysiology of AD (Ginsberg *et al*, 2000,2004,2006b).

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Genes changed within neurofibrillary tangles—Histopathological and biochemical studies show that hippocampal CA1 pyramidal neurons of the AD brain bear intracellular NFTs, which have been modeled in mice that overexpress the human t protein (Ginsberg *et al*, 1999b; Trojanowski and Lee, 2005; Gotz, 2001; Andorfer *et al*, 2005). Microaspiration coupled with microarray analysis was used to uncover mechanisms that might underlie the formation of NFTs (Ginsberg *et al*, 2000, 2004, 2006b). Relative to non-tangle-bearing CA1 neurons obtained from normal control brains, neurons harboring NFTs in AD brains displayed reductions in mRNAs for cytoskeletal elements, dopamine (DA) receptors, glutamate receptors (GluRs), protein phosphatase subunits 1α and 1γ, kinases, and synaptic-related markers (Ginsberg *et al*, 2000, 2004; Ginsberg, 2007). In addition, microarray analysis has demonstrated that protein phosphatase 3CB, also known as calcineurin Aβ, is upregulated in NFT-bearing neurons within the AD hippocampus (Hata *et al*, 2001).

The expression of D1–D5 subtypes of DA receptors, particularly D2, and the DA transporter (DAT) are decreased by 2- to 4fold in NFT-bearing neurons in AD *vs* non-tangle-bearing neurons in control brains, mRNA (Figure 6) (Ginsberg *et al*, 2000,2004). These findings are consistent with the ability of D2 receptor agonists to reverse motor impairments associated with normal aging in rodents (Altar and Marshall, 1988), and decreased D2 radioligand binding in AD hippocampus (Joyce *et al*, 1993;Ryoo and Joyce, 1994). A variety of behavioral impairments associated with dopaminergic dysfunction are seen in AD patients, including impaired motor function, anxiety, cognitive decline, hallucinations, and psychosis. The treatment of AD patients with DA antagonists such as risperidone has become more common, though controversial, in care facilities for AD patients (Katz *et al*, 2007). The decreases in DA receptor expression and ligand binding in these patients may be counterintuitive to the use of D2 antagonists, and warrants a closer scrutiny for the basis of their use, or the alternative use of D2 agonists, such as the mixed D2/D3 agonist piribedil, which enhances cognitive skill learning in healthy older adults (Peretti *et al*, 2004).

Glutamatergic dysfunction in AD has been assessed at the gene and protein level for two decades. One of the few FDA-approved medications for cognitive decline in AD is memantine, a non-competitive antagonist of *N*-methyl-ᴅ-aspartate (NMDA) receptors (Lipton, 2007). Independent microarray evaluations indicate that hippocampal α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (GRIA1 and GRIA2, corresponding to AMPA1 and AMPA2, respectively) and NMDA receptor (eg, GRIN1) subunits, and select glutamate transporters (eg, SLC1A1, or EAAT2, a neuronal specific glutamate transporter) are downregulated during the progression of AD, consistent with earlier pharmacological binding, immunoblot, and immunocytochemical evaluations (Ginsberg, 2007; Ginsberg *et al*, 2000, 2006b; Westphalen *et al*, 2003; Jacob *et al*, 2007; Yasuda *et al*, 1995; Ikonomovic *et al*, 1995; Carter *et al*, 2004). The vulnerability of cortical and hippocampal glutamatergic synaptic machinery, including these ionotropic GluRs and glutamate transporters, is consistent with the roles that excitotoxic mechanisms have been proposed to play in the pathogenesis of AD (Ginsberg *et al*, 1999b; Trojanowski and Lee, 2005; Hyman *et al*, 1984; Carter *et al*, 2004), and merit further consideration as drugable targets (Rothstein, 2003).

Genes related to synaptic function, particularly those for presynaptic vesicles and postsynaptic docking machinery, are another class of genes shown by microarray analysis to be relevant to AD pathogenesis (Ginsberg, 2007; Loring *et al*, 2001; Miller *et al*, 2008; Ginsberg *et al*, 2000; Yao *et al*, 2003; Blalock *et al*, 2003). Indeed, the losses of synapses and the nerve terminal-specific protein, synaptophysin (SYP), correlate better with cognitive decline than do increases in amyloid load or τ pathology (Heffernan *et al*, 1998; Shimohama *et al*, 1997; Sze *et al*, 1997, 2000). Decreased synapse-related markers are consistently observed in NFT-bearing CA1 neurons. These include SYP, synaptotagmin I, synapsin I (SYN I), α-synuclein, and β-synuclein (Ginsberg *et al*, 2000, 2004, 2006b) (Figure 7).

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Decreases in SYN I, II, and III were also found in the entorhinal cortex of AD patients (Ho *et al*, 2001). SYP decreases in AD hippocampus have been confirmed by qPCR and *in situ* hybridization (Gutala and Reddy, 2004; Callahan *et al*, 1999). These and other genes that encode for synapse-specific proteins may help evaluate the efficacy of therapeutic compounds in animal models, postmortem human tissues or in patients, based on the use of imaging biomarkers.

Genes changed within cholinergic basal forebrain neurons—Degeneration of the cholinergic basal forebrain (CBF) system is an early event in AD pathogenesis, and probably contributes to the cognitive abnormalities seen in MCI and AD (Mufson *et al*, 2003; Whitehouse *et al*, 1982; Bartus, 2000). One likely suspect for CBF neurodegeneration is nerve growth factor (NGF), a neurotrophin (NT), the administration of which, similar to that of brain-derived neurotrophic factor (BDNF), or NT-3, promotes their survival in a variety of animal lesion models. NGF is synthesized and secreted by cortical and hippocampal cells, and binds to the high-affinity NGF receptor, trkA, and low-affinity NGF receptor, $p75^{NTR}$ that are highly enriched in somatic and synaptic sites within CBF neurons (Mufson *et al*, 2003, 2007a; Sofroniew *et al*, 2001).

Individual cholinergic neurons collected from the nucleus basalis of AD patients were evaluated with custom-designed cDNA arrays (Figure 2) and changes were verified with real-time qPCR and *in situ* hybridization. CBF neurons displayed decreases in the expression of the receptors for NGF, BDNF, and NT-3, namely, TrkA, TrkB, and TrkC, respectively (Ginsberg *et al*, 2006b,c;Counts *et al*, 2007). Moreover, an EST targeted to the extracellular and tyrosine kinase domains were decreased for each Trk gene (Ginsberg *et al*, 2006c). These results have been validated by qPCR in the basal forebrain of AD patients and are consistent with *in situ* hybridization studies of Trk receptors (Ginsberg *et al*, 2006c;Boissiere *et al*, 1997). Moreover, increases in the expression of the α-7 nicotinic acetylcholine receptor (CHRNA7) were seen during the progression of AD in these studies. Interestingly, an intermediate reduction in the NT receptors was observed in MCI. In contrast, mRNAs for $p75^{NTR}$ choline acetyltransferase (ChAT), the other nicotinic and muscarinic acetylcholine receptor subunits, or GAPDH, were not changed across clinical conditions (Ginsberg *et al*, 2006b,c;Counts *et al*, 2007). These decreases in TrkA, TrkB, and TrkC mRNA during prodromal stages of AD are associated with cognitive decline, as measured by a Global Cognitive Score and the Mini-Mental State Examination (Ginsberg *et al*, 2006c;Mufson *et al*, 2007b). These results suggest that the decreases in high-affinity NT receptor expression in CBF neurons occur during the early stages of cognitive decline and are associated with the clinical presentation of the disease. Thus, Trk defects may be molecular markers for the transition from normal cognitive function to MCI, and from MCI to AD. Interestingly, recent SNP analysis of the CHRNA7 haplotype in a large population of aged subjects concluded that genetic variation in CHRNA7 influences susceptibility to AD (Carson *et al*, 2008). These results also provide support for the development of agonists and/or modulators of CHRNA7 as a potential pharmacotherapeutic treatment for AD.

On the basis of animal model and clinical studies, the delivery of NGF (Mufson *et al*, 2007a; Bartus, 2000; Tuszynski, 2007) or BDNF (Morse *et al*, 1993) has been proposed as treatments to prevent or delay the onset of AD. There are significant limitations for NGF or BDNF delivery to the CNS, including their failure to cross the blood–brain barrier and, during intracranial delivery, poor bioavailability, poor bioavailability, their *in vivo* instability, and proliferative effects (Bartus, 2000; Kaplan and Miller, 2000). CNS transplantation with cells that secrete NTs is technically challenging and faces the same limitations as infused NTs. Because of these limitations, small molecule partial agonist activators of Trk receptors have been designed and tested for the treatment of AD (Skaper, 2008; Mocanu *et al*, 2008). A high-throughput screening assay of small-molecule agonists

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for the TrkA receptor has identified gambogic amide, an alkaloid used in traditional Chinese medicine (Jang *et al*, 2007). It selectively binds TrkA (but not TrkB and TrkC), phosphorylates TrkA tyrosine residues, and activates the Akt and MAPK TrkA-mediated NGF signaling pathways. Gambogic amide attenuates excitotoxic damage to cells and promotes neurite outgrowth in PC12 cells (Jang *et al*, 2007), making this a lead for chemical modification and potential for AD clinical trials.

No mutations have been described in the low molecular weight microtubule-associated protein τ, the principal component of NFTs in AD (Ginsberg *et al*, 1999b; Hyman and Trojanowski, 1997; Mirra *et al*, 1991). However, mutations in the τ gene cause frontotemporal dementia (Goedert and Jakes, 2005), suggesting that post-transcriptional alterations in τ expression may contribute to tauopathies, including AD and related dementing disorders. Gene expression profiling within single nucleus basalis and CA1 hippocampal neurons revealed an increase in the expression of the three-repeat τ (3Rτ) to four-repeat τ (4Rτ) mRNAs during the progression of AD, but not during normal aging (Ginsberg *et al*, 2006a). An increase in the proportion of 3Rτ/4Rτ mRNA was found in MCI and AD relative to those with normal cognition (Ginsberg *et al*, 2006c), suggesting that this dysregulation impacts neuronal function and marks a transition from normal cognition to prodromal AD. An increase in the 3Rτ/4Rτ expression ratio may increase neuronal vulnerability, a model worth further evaluation as agents that regulate this pattern of τ expression might be beneficial.

Genes changed within Senile plaques—The biochemical composition of SPs is well known, but little data exist on the non-proteinaceous components of these lesions. The presence of mRNA, rRNA, and tRNA was evaluated in SPs using AO histofluorescence, alone or in combination with thioflavine-S staining and immunocytochemistry in AD brains and related neurodegenerative disorders with abundant SPs (Ginsberg *et al*, 1997, 1998). Quantitative analyses demonstrated that about 55% of thioflavine-S-stained SPs also contain AO histofluorescence (Ginsberg *et al*, 1997, 1998), indicating that RNA species are localized to a significant population of SPs. The sequestration of RNAs prompted single-cell gene expression analysis of SPs in AD hippocampus. Expression profiles of SPs were compared with individual CA1 neurons and surrounding neuropil of control brains (Ginsberg *et al*, 1999a). Results indicate that the expression profiles amplified from SPs are predominantly neuronal. SPs harbor two distinct populations of expressed genes. One cluster of transcripts contains high abundance genes including amyloid-β precursor protein (APP), bax, Bcl-2, AMPA GluR subunits and τ (Ginsberg *et al*, 1999a, 2004). A second grouping of transcripts contains low abundance genes including glial-enriched mRNAs such as glial fibrillary acidic protein (GFAP), interleukin-1 (IL-1), and the receptor for advanced glycation end products (AGER) (Ginsberg *et al*, 1999a, 2004). Combined single-cell expression profiling, *in situ* hybridization, and PCR data sets indicate that multiple mRNA species are found in individual, extracellular SPs. SPs appear to sequester the remnants of degenerating neurons and their processes, including mRNA, and these properties can be studied in animal models of cerebral amyloid overexpression (Dickey *et al*, 2003; Reddy *et al*, 2004).

Parkinson's disease—PD is the second most frequently occurring age-related chronic neurodegenerative disease, with both common sporadic and rare familial forms. Clinical symptoms include resting tremor, bradykinesia, hypokinesia, and postural disturbances. Key pathological features are the loss of dopaminergic neurons within the substantia nigra pars compacta (SNPC), loss of striatal DA, and other neurotransmitters including serotonin and norepinephrine, and the presence of inclusions known as Lewy bodies that consist of α-synuclein (Fearnley and Lees, 1991).

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Genes changed in PD—Expression profiling studies have revealed downregulation of genes critical to mitochondrial function and energy synthesis and maintenance, supporting several lines of evidence from animal models that experimental perturbations such as complex I inhibitors of the respiratory chain and mitochondrial poisons result in a PD-like pathology in the SNPC (Table 1) (Mandel *et al*, 2003;Grunblatt *et al*, 2001;Greene *et al*, 2005;Napolitano *et al*, 2002;Patel *et al*, 2008). Mechanisms underlying these reductions may involve mitochondrial function itself, which increases reactive oxidative species and promote oxidative stress and mitochondrial failure, leading to synaptic loss and neurodegeneration, which have also been reflected in transcript alterations in microarray analysis of PD (Miller *et al*, 2006;Vogt *et al*, 2006).

Microarray analysis of RNA harvested from the SNPC of PD cases identified decreased transcripts for the ubiquitin–proteosome system (eg, SKP1, UCHL1) and ion transport (eg, EGLN1), among others (Grunblatt *et al*, 2004). Another microarray analysis of postmortem SNPC tissue from PD patients identified increases in other ubiquitin–proteosome system components when compared with age-matched controls, including the 70 kDa heat-shock protein (HSPAlA) (Hauser *et al*, 2005). A twofold decrease in the expression of the *PARK5* familial PD-linked ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) gene is observed within the SNPC of PD patients (Liu *et al*, 2002), and a proteomic assessment in postmortem PD brains found downregulation of UCHL1 protein (Choi *et al*, 2004). Moreover, UCHL1 itself is a target for protein oxidation (Choi *et al*, 2004), suggesting a link among mitochondrial dysfunction, oxidative stress, and reduced proteasomal activity, all of which have been implicated in several recent microarray studies in rodent and non-human primate models of PD (Greene *et al*, 2005; Iwata *et al*, 2007; Duke *et al*, 2006; Chin *et al*, 2008; Miller *et al*, 2004). These observations suggest that a common final pathway for buffering cellular toxicity of misfolded proteins may be dysfunctional in PD. Interestingly, transcripts and their expressed proteins involved in ubiquitin–proteasome and mitochondrial functions are also downregulated in two models of PD, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine delivery (Chin *et al*, 2008), further validating the human microarray studies and lending additional genomic and proteomic credence to the use of these versatile models for the assessment of molecular and cellular pathogenesis in PD.

Genes changed by anti-Parkinsonian therapies—It is interesting that over half of all PD patients receiving electroconvulsive therapy (ECT) for psychiatric depression also show improvement in Parkinsonian symptoms (Faber and Trimble, 1991). The mechanism of this anti-Parkinsonian effect of ECT is unknown; however, microarray profiling in a rodent model of ECT, electroconvulsive shock (ECS), reveals ECS increases in genes that could ameliorate DA deficiencies (Altar *et al*, 2004; Newton *et al*, 2003). Nurr1, which is elevated after acute and chronic ECS (Altar *et al*, 2005), is an orphan nuclear receptor required for the differentiation of mid-brain dopaminergic neurons. Hemizygous Nurr1 deletions decrease limbic and cortical DA levels (Eells *et al*, 2002). Nurr1 expression is decreased in nigral neurons that contain Lewy bodies in PD brains (Chu *et al*, 2006). ECS also increases BDNF, TrkB, early growth response 1 (EGR1), glutathione *S*-transferase, *S*-adenosylmethionine decarboxylase, arc, fibroblast growth factor (FGF) receptor-1, heme oxygenase-3, and S100 calcium-binding protein, in addition to Nurr-1 (Altar *et al*, 2004, 2005). The protein products of each of these genes could enhance DA functions or neuronal protection in PD.

Few studies have employed microarrays to profile mRNA changes to L-dopa, DA agonists, or other drugs or surgical approaches used to treat PD. Such studies would be of interest as they may identify gene overlaps with those that change in response to ECS or those that are reciprocally changed in PD. One such gene is Nurr-1, which increases in SH-SY5Y

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neuroblastoma cells exposed to the D2/3 receptor agonist, pramipexole, as do DAT and VMAT2 (Pan *et al*, 2005). Interestingly, the Nurr-1 increase is mediated by the D3 receptor, which can be blocked by nafadotride, a D3 receptor antagonist (Pan *et al*, 2005). Another overlapping gene is EGR1, the gene and protein products of which are increased in rat frontal cortex by 4-week treatment with Abilify, a partial D2 agonist (Cheng *et al*, 2008).

Rats with extensive dopaminergic lesions induced by neurotoxins including 6-hydroxydopamine (6-OHDA) or MPTP have been evaluated for specific striatal gene expression changes following L-dopa. In most cases, L-dopa challenge was associated with the production of dyskinesias or self-injurious behaviors and those studies will not be summarized here. More moderate, and subchronic, doses of L-dopa that more closely model anti-Parkinsonian treatment regimens induce a variety of mRNA changes in the lesioned hemisphere. These include increases in the receptor activity-modifying protein, RAMP1 in striatum (Lee *et al*, 2008), the vesicular GABA transporter (vGAT) in the striatonigral pathway (Wang *et al*, 2007a), striatal glutamic acid decarboxylase 67 (GAD67), but not GAD65, dynorphin, enkephalin (Tronci *et al*, 2007), secretogranin II, preproenkephalin A, secretoneurin (Medhurst *et al*, 2001), c-jun and c-fos (Svenningsson *et al*, 2002), and a reversal of the decrease in striatal Src, Lyn and PKC expression induced by 6-OHDA (Napolitano *et al*, 2006). Cannabinoid receptor 1 was another gene among the 10 genes increased in rats by the D2 partial agonist Abilify (Cheng *et al*, 2008), and is increased in the 6-OHDA striatum by L-dopa (Zeng *et al*, 1995). The pattern of mRNA changes after other D2 agonists, such as apomorphine, pergolide, bromocriptine, and SKF 38393 are far less studied and complex (Granata *et al*, 1996). They appear to preferentially affect the DA-denervated striatum, possibly due to D2/D3 receptor supersensitivity. More analysis is needed to relate changes in striatal and nigral gene expression to this class of drugs that account for differences in their degree of agonism, tolerance, and pharmacokinetic properties.

A candidate approach for treating PD, adenosine A(2A) receptor antagonism, potentiates L-dopa-induced c-fos expression and contraversive rotations (Fenu *et al*, 1997). A(2A) antagonism also reverses the increased expression of PPE in 6-OHDA-denervated rat striatum, thus demonstrating that this approach normalizes the activity of striato-pallidal enkephalin-containing neurons (Aoyama *et al*, 2002). The upregulation of c-fos by ECS and by L-dopa and A(2A) antagonism in PD lesion models is consistent with a PKC- or PKA-mediated activation of gene expression in the treatment of PD (Figure 13).

Normal Aging

The longevity of a given species is a product of several parameters, including frailty (ie, intrinsic vulnerability to death) and senescence (ie, the rate of change in frailty over time) (Gems and McElwee, 2003; de Magalhaes, 2004). About half of the variation in human lifespan is attributable to genetic variation (Pletcher and Stumpf, 2002), but individual genes directly responsible for these aging events have been difficult to identify. Telomere shortening with age reduces the proliferative capacity of somatic cells and has been linked to premature aging (Zucchero and Ahmed, 2006). Other genes appear to underlie aging in invertebrate and mammalian systems, where mutations in genes including daf-2, daf-16, and IGF-1 have been shown to extend lifespan (Vijg and Suh, 2005; Kirkwood, 2005; Golden and Melov, 2004; Kenyon *et al*, 1993; Chen *et al*, 2007). The majority of these genes encode protein regulators or modifiers of energy utilization. In contrast, mutations in genes that encode proteins responsible for DNA repair or cell cycle control accelerate the aging process (Vijg and Suh, 2005). Association studies have used population-based genomic approaches to compare the prevalence of genetic markers between long-lived and randomly chosen individuals (Hauser and Pericak-Vance, 2000). Potential markers are then identified by linkage disequilibrium, the nonrandom inheritance of alleles located in proximity to each

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other in the genome. The prevalence of a marker in long-lived individuals is evidence that the marker may either be a causal genetic variant or linked nearby to a causal variant. Fortunately, high-throughput technologies have enabled the creation of public databases that annotate relevant genes and expression profile data in aging paradigms. For example, the Human Ageing Genomic Resources (HAGR) provides online resources for the biology of aging (http://genomics.senescence.info) (Raghothama *et al*, 2005; de Magalhaes *et al*, 2005). Several databases are available for public queries on nematode aging including WormBase (http://wormbase.org) (Hunt-Newbury *et al*, 2007; Rogers *et al*, 2008).

Oxidative stress—Age-related accumulation of reactive species of oxygen and nitrogen, which increase lipid peroxidation, and heightened oxidative damage, have been well documented (Sohal and Weindruch, 1996; Barja, 2004; Serrano and Klann, 2004). The aging brain is associated with heightened apoptosis and immune activity that lead to the accumulation of misfolded proteins, which confer their own set of negative sequelae to affected cells. Microarray studies have identified altered regulation of markers of oxidative damage during the aging process in humans and animal models (Blalock *et al*, 2003, 2004; Reddy *et al*, 2004; Prolla, 2002; Lee *et al*, 2000; Brooks *et al*, 2007). An Affymetrix array analysis of frontal cortex of 30 normal male subjects aged from 26 to 106 years indicated that mRNA expression profiles were most consistent for subjects <40 years old, with another relatively homogeneous expression profile occurring in subjects >70 years old (Lu *et al*, 2004). The greatest variability in expression levels was found in the 40- to 70-year-old subjects, suggesting that the rate of change in expression levels advances throughout middle age. The middle age and aged groups showed an upregulation of cortical genes and pathways related to oxidative damage and inflammation, and downregulation of genes associated with DNA repair and synaptic function, particularly for vesicular transport and neurotransmission (Miller *et al*, 2008; Blalock *et al*, 2003; Brooks *et al*, 2007; Lu *et al*, 2004). Promoter analysis assays and evaluation of RNA structure demonstrated oxidative damage to DNA and RNA during senescence (Lu *et al*, 2004; Abe *et al*, 2002; Shan *et al*, 2003), and abnormal processing of proteins has been observed from oxidized mRNAs expressed *in vitro* (Shan *et al*, 2003). Importantly, microarray analyses in AD, PD, and motor neuron disease have consistently shown dysregulation of genes related to oxidative stress (Altar *et al*, 2005; Blalock *et al*, 2003; Grunblatt *et al*, 2004; Brooks *et al*, 2007; Manczak *et al*, 2004; Matzilevich *et al*, 2002), making this class of transcripts one of the most important to evaluate for therapeutic interventions in aging paradigms. The products of these genes can help scavenge free radicals and protect cellular membranes, avoid DNA and RNA damage, and promote the DNA/RNA repair mechanisms (Galvin and Ginsberg, 2005).

DA receptors—Single-cell RNA was amplified and evaluated by custom-designed cDNA arrays for changes in over 200 genes in hippocampal CA1 pyramidal neurons and entorhinal cortex layer II stellate cells from normal persons who died at 19–95 years of age (Hemby *et al*, 2003). A dramatic age-related decline was found for all five DA receptor mRNAs in CA1 pyramidal neurons, but not in entorhinal cortex stellate cells (Figure 7) (Ginsberg *et al*, 2004; Hemby *et al*, 2003). No age-related decrement in other mRNAs was observed in CA1 pyramidal neurons, including the cytoskeletal elements β-actin, 3Rτ, or 4Rτ (Hemby *et al*, 2003) (Figure 7).

Alterations in hippocampal DA function impact cognitive and mnemonic functions, and disruption in the functional integrity of hippocampal DA neurotransmission correlates with the pathophysiology of neurodegenerative disorders including AD and neuropsychiatric disorders such as schizophrenia. Deficits in dopaminergic neurotransmission may also contribute to cognitive decline associated with normal human aging and in animal models (Volkow *et al*, 1996; Rinne *et al*, 1990; Amenta *et al*, 2001). Aging may be a factor responsible for cell-type-specific downregulation of many genes (Lu *et al*, 2004), with

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downregulation of DA receptor gene expression contributing to deficits in learning and memory, anxiety, and depressive-like syndromes during aging. It is of interest that the D2/D3 agonist piribedil, which increases cognitive skill learning in aged adults (Peretti *et al*, 2004), restores the 21% decrease in brain protein synthesis rates in aged rats as compared with those of middle-aged rats (Bustany *et al*, 1995).

Psychiatric Indications

Schizophrenia—Schizophrenia is a severe and life-long psychiatric disorder characterized by illogical, delusional, or paranoid thoughts (positive symptoms), and cognitive deficits, including impairments in working memory, attention, and executive function. The positive symptoms constitute the more overt manifestations of psychosis and are typically the first to draw attention to the disorder. The cognitive components are perhaps more variable, yet more enduring, and, in many respects, are more disabling. Negative symptoms, including social withdrawal, flattened affect, and decreased initiative, characterize most schizophrenic patients to some extent, and constitute the majority of symptoms in about 15% of schizophrenics (Bleuler, 1950; Kraeplin, 1919).

Similar to most psychiatric illnesses, neither the cause nor the mechanism(s) underlying the pathophysiology of schizophrenia are clear, and much of what is known is inferred from the response, or non-response, of patients to pharmacologic therapies. Most antipsychotic drugs are more effective in ameliorating the positive symptoms than the negative symptoms (Altar *et al*, 2003a). Examples include the 'typical' antipsychotics haloperidol and chlorpromazine, and the 'atypical' antipsychotics clozapine, thioridazine, olanzapine, ziprasidone, and aripiprazole, which produce fewer adverse side effects, such as Parkinsonian-like symptoms (Altar *et al*, 2003a; Taylor *et al*, 2008). Atypical antipsychotics may improve cognitive deficits, although as determined by a meta-analysis of neuropsychological change to clozapine, olanzapine, quetiapine, and risperidone in schizophrenia, the effects are small (Woodward *et al*, 2005). The following summarizes genes and functional classes of transcripts associated with schizophrenia and potential targets for therapeutic development.

Gene changes in schizophrenia brain—Several groups initially compared the gene expression profiles in postmortem dorsolateral prefrontal cortex (DLPFC) dissected *en bloc* from control subjects and age- and sex-matched schizophrenic cases (Vawter *et al*, 2001, 2002a, 2004b; Mirnics *et al*, 2000; Middleton *et al*, 2002; Hakak *et al*, 2001). Not a single gene change was consistently replicated among all three groups, and several genes including malate dehydrogenase 1 cytosolic (MDH1) and SERPINI1 (neuroserpin) were found to change in opposite directions (Hakak *et al*, 2001; Vawter *et al*, 2002a). Gender differences in the expression of MDH1 and neuroserpin may have contributed to this result, as both genes are more highly expressed in male subjects as compared with female subjects with schizophrenia and in women *vs* men among the controls (Vawter *et al*, 2004a).

Subsequent microarray studies of schizophrenia incorporated improvements including better case–control matching, and validation with qPCR, *in situ* hybridization, and SNP analysis (Altar *et al*, 2005; Arion *et al*, 2007; Aston *et al*, 2004; Haroutunian *et al*, 2007; McCullumsmith *et al*, 2007; Hashimoto *et al*, 2008; Mexal *et al*, 2005; Middleton *et al*, 2005; Mirnics *et al*, 2001; Katsel *et al*, 2005a; Iwamoto *et al*, 2005; Iwamoto and Kato, 2006; Shao and Vawter, 2008; Saetre *et al*, 2007; Benes *et al*, 2007). These studies revealed consistently modest effect sizes (20–40%), but statistically significant changes for genes in multiple cortical and hippocampal regions. Many abundantly expressed genes showed robust, co-directional changes in schizophrenia when compared with controls in two or more studies (eg, UCHL1, GAD1, GAD2, interferon-induced transmembrane protein 3 (IFITM3), MAG, MDH1, SYN3, TF, YWHAH, and RGS4) (Table 2). Changes in some genes were

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confirmed by other techniques such as proteomics (Table Box 2), *in situ* hybridization, or RT-PCR. The subtraction of the estimated effects of comorbid alcohol and substance abuse, gender, age, and pH on gene expression reduced the number of significant genes for schizophrenia (Shao and Vawter, 2008). Nevertheless, decreases in prefrontal cortex and cerebellar GAD67 protein and mRNA were replicated in patients with schizophrenia or bipolar disorder (BPD) with psychosis, and these decreases were unrelated to postmortem intervals, dose, duration, or presence of antipsychotic medication (Guidotti *et al*, 2000). Decreases in genes related to mitochondria, oligodendroglia–myelin pathways, metabolism, ubiquitin–proteasome function, chaperone functions, and immune responses and apoptosis pathways were confirmed in prefrontal cortex (Hakak *et al*, 2001; Arion *et al*, 2007; Haroutunian *et al*, 2007; Shao and Vawter, 2008; Saetre *et al*, 2007; Tkachev *et al*, 2003; Shao *et al*, 2008) and hippocampal dentate granule neurons (Altar *et al*, 2005). These initial expression studies showed that disease-specific effects would require the analysis of larger sample numbers, additional brain regions, and possibly more homogeneous tissue or cell-specific sampling. Prominent and concordant gene expression differences found in at least two microarray studies of schizophrenia are described in the following sections (Table 2).

HINT1 (histidine triad nucleotide-binding protein 1), parvalbumin, and GAD67 —HINT1 gene expression was decreased in three postmortem schizophrenia microarray studies (Vawter *et al*, 2001, 2002a; Ryan *et al*, 2006). *In situ* hybridization showed HINT1 to be reduced by 35% in cortical layer VI and by 21% in layer II–V neurons (Vawter *et al*, 2004b). Interestingly, HINT1 is co-expressed in parvalbumin-containing interneurons (Liu *et al*, 2008), and parvalbumin expression is decreased in schizophrenia prefrontal cortex and correlates strongly with the decreased densities of GAD67 mRNA-positive neurons (Hashimoto *et al*, 2003, 2008; Lewis and Moghaddam, 2006). Either clozapine or haloperidol increases GAD67 expression, as shown by ISH in rat neocortex (Zink *et al*, 2004). An HINT1 knockout mouse model displays mania-like behaviors, and is more behaviorally responsive to amphetamine than are wild-type mice (Barbier *et al*, 2007). The normal presence of HINT1 in human and animal GABAergic basket interneurons (Liu *et al*, 2008; Barbier *et al*, 2007) may explain its reduction in schizophrenia, as the immunoreactivity of parvalbumin and the GABA membrane transporter, GAT2-containing basket, and chandelier cells in layers III and IV are decreased in schizophrenia (Beasley and Reynolds, 1997). HINT1 is a member of the histidine triad superfamily of nucleotide hydrolases (Brenner, 2002), complexes with transcription factors MITF or USF2 and lysyl-tRNA synthetase, and lysyl-AMP may be a native substrate for HINT enzymes (Chou and Wagner, 2007). HINT1 may be involved in postsynaptic phosphorylation of neurotransmitter receptors (Guang *et al*, 2004).

14-3-3 gene family—The 14-3-3 proteins are involved in intracellular signaling, cell division, cell differentiation, apoptosis, ion channel functioning, and neurotransmission (Berg *et al*, 2003). Their first mode of action was identified with serotonin–*N*-acetyltransferase, a pineal gland enzyme that is catalytically inefficient for melatonin synthesis. After phosphorylation, however, the 14-3-3 protein binds to and stabilizes the enzyme's active conformation (Ganguly *et al*, 2001; Obsil *et al*, 2001). It was then discovered that the 14-3-3 proteins also activate tyrosine hydroxylase, tryptophan hydroxylase (TPH), and inhibitors of protein kinase C, including HINT1 (Bridges and Moorhead, 2005). In rat brain, 14-3-3 family members associate and colocalize with GABA receptors (Couve *et al*, 2001), disrupted in schizophrenia- 1 (DISC1) (Taya *et al*, 2007), CHRNA α4 subunit (Jeanclos *et al*, 2001), and TPH2 (Winge *et al*, 2008). There are seven 14-3-3 genes: β (YWHAB), ε (YWHAE), η (YWHAH), γ (YWHAG), σ (YWHAS), θ (YWHAQ), and ζ (YWHAZ). The 14-3-3 proteins are present in synaptic membranes and isolated synaptic junctions and may influence neurotransmission by regulating exocytosis or

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phosphorylation of synaptic proteins (Martin *et al*, 1994), including the NMDAR2A receptor by YWHAH (Pozuelo Rubio *et al*, 2004).

The 14-3-3 gene expression studies indicate that YWHAB, -E, -G, -H, -Q, and -Z are decreased in the DLPFC of schizophrenia cases (Table 2). Drug interactions with specific 14-3-3 family members may have antipsychotic benefits. Mechanisms to increase 14-3-3 may include the use of acetyl-L-carnitine, which increases YWHAG (Traina *et al*, 2004), or fluoxetine, which induces YWHAZ and YWHAE (Cecconi *et al*, 2007). Haloperidol increased YWHAB in monkey DLPFC and but does not alter YWHAG or YWHAZ in rat brain (Wong *et al*, 2003;Dean *et al*, 2007). It will be of interest to screen antipsychotic drugs for their effects on the 14-3-3 subtypes. Decreases in the η protein (YWHAH) were also found in the middle temporal gyrus and cerebellum of schizophrenia cases (Vawter *et al*, 2001) and confirmed in DLPFC in two other cohorts of schizophrenics (Middleton *et al*, 2005;Iwamoto *et al*, 2005). Interestingly, the YWHAH gene is located at 22q12.2, close to the DiGeorge region 22q11.2. This deletion is the next highest risk factor for schizophrenia after having a schizophrenic identical twin, and shows a significant association with schizophrenia for two polymorphisms (Wong *et al*, 2003). Altogether, these studies indicate that YWHAH, YWHAB, YWHAE, YWHAG, YWHAQ, and YWHAZ are decreased in the DLPFC of schizophrenia. The YWHAE protein was decreased in the DLPFC of two cohorts of schizophrenic cases (Novikova *et al*, 2006), reinforcing the mRNA decreases in this gene family (Vawter *et al*, 2001,2002a;Middleton *et al*, 2005).

YWHAH and YWHAZ are reported to be susceptibility genes for schizophrenia (Bell *et al*, 2000; Jia *et al*, 2004; Toyooka *et al*, 1999; Wong *et al*, 2005), but three additional studies provide no such evidence for YWHAH (Wang *et al*, 2005; Hayakawa *et al*, 1998; Duan *et al*, 2005). A resequencing of YWHAH, YWHAZ, and other 14-3-3 proteins for more rare polymorphisms may determine whether functional polymorphisms are associated with schizophrenia. Interestingly, a systems biology approach identified YWHAZ to be decreased in frontal neocortex of aging and AD cases, and that it is a ‘hub gene’ candidate for further analysis in the pathogenesis of age-related neurodegeneration (Miller *et al*, 2008).

Synapse-related genes—Decreases in genes that encode for synaptic-related markers (Mirnics *et al*, 2000; Vawter *et al*, 2002b) were initially reported for SYN2, NSF, GRIA2, and SYT5, but were not found in more recent studies that used larger commercial microarrays and better parametric statistical approaches. SNP or protein confirmation has nonetheless been obtained for SYN II (Lee *et al*, 2005; Saviouk *et al*, 2007), ionotropic GluR AMPA2 (GRIA2) (Beveridge *et al*, 2008), and GAD67 (GAD1) (Guidotti *et al*, 2000; Hashimoto *et al*, 2003; Straub *et al*, 2007; Addington *et al*, 2005). The use of dissected brain blocks may explain the lack of additional confirmation for decreases in SYN II reported in earlier microarray studies (Gray *et al*, 2006; Imai *et al*, 2001).

GABAergic genes—An analysis of all postmortem studies of schizophrenia conducted in specimens from the Stanley Neuropathology Consortium revealed that three genes expressed in GABA neurons, namely reelin (RELN), parvalbumin (PV) and GAD1, had the most abnormal transcript and protein levels (Torrey *et al*, 2005). GAD1 and other markers of GABAergic neurotransmission are also downregulated in DLPFC, as determined by microarray analysis (Hashimoto *et al*, 2008). The cellular specificity of gene expression changes in schizophrenia was reinforced by the large and highly significant decreases in GAD1 expression found in the stratum radiatum, pyramidale, and oriens (Benes *et al*, 2007), but not in whole hippocampus (Konradi *et al*, 2004). GAD2 is decreased in stratum oriens (Benes *et al*, 2007) and GAD1 is decreased in schizophrenia neocortex (Mirnics *et al*, 2000; Vawter *et al*, 2001; Akbarian *et al*, 1995). As noted in this review, some inconsistencies in

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gene changes between microarray studies probably resulted from between-study differences in the gender of samples or agonal state (Lewis and Moghaddam, 2006).

Protein changes in schizophrenia brain—Some microarray-based mRNA changes in schizophrenia DLPFC have been confirmed by proteomic studies conducted in the same region. We compared two proteomic studies of frontal cortex in schizophrenia (Prabakaran *et al*, 2004; Behan *et al*, 2008) in which about 119 proteins were changed in either study. Confirmation of many of those protein changes, particularly in metabolic, immune, and oligodendroglia groups, were provided by up to three expression studies (Table Box 2). The protein glutamate-ammonia ligase (GLUL; Figure 10) was also decreased in Brodmann areas 10 (BA 10) (Burbaeva *et al*, 2003) and BA 9 (Prabakaran *et al*, 2004) in schizophrenia. Interestingly, multiple thalamic nuclei showed increased expression of the GLUL transcript (Bruneau *et al*, 2005). In addition, four proteins (α-enolase 1, peroxiredoxin-2, fructose bisphosphate aldolase C and dihydropyriminidase-related protein 2) decreased in both proteomic studies, and brain acid-soluble protein 1 increased in both studies (Table Box 2).

Genes for synaptic proteins were decreased in schizophrenia neocortex (Mirnics and Lewis, 2001), and comprised 7 of 15 genes, the protein levels of which were changed in another cohort (Mirnics and Lewis, 2001; Pennington *et al*, 2007). Metabolic- or mitochondrial-associated proteins comprised 25 of the 51 genes that were changed in BPD (Middleton *et al*, 2002; Pennington *et al*, 2007) and these decreases in functional gene classes were also found by Middleton *et al* (2002).

A more comprehensive survey of gene changes in schizophrenia was provided by the SMRI gene expression database (Elashoff *et al*, 2007) (http://216.55.162.172/in-dex.html). The SMRI combined and analyzed multiple microarray investigations of the DLPFC from schizophrenia, BPD, and control cases provided by the groups of CA Altar, S Bahn, H Chen, SE Dobrin, A Fienberg, T Kato, P Sklar, MP Vawter, and LT Young. A relative risk score for the over-representation of genes in each disease and pathway was used to rank pathway terms for their disease association. Pathways significant in the SMRI genomic database (at a false discovery of $p = 0.05$) using adjusted individual microarray studies (Higgs *et al*, 2006) for schizophrenia include: generation of Aβ peptides by presenilin 1 (PS1), regulation of MAP kinase pathways through dual specificity phosphatases, oxidative phosphorylation, ATP synthesis, and ribosomal pathways. A compelling mediator of the MAP kinase could be BDNF, which, along with its high-affinity receptor, TrkB, is expressed at lower levels in schizophrenia prefrontal cortex, as determined by *in situ* hybridization of different cohorts (Weickert *et al*, 2003; Hashimoto *et al*, 2005). Decreases in BDNF were localized to large pyramidal neurons in cortical layers III, V, and VI (Weickert *et al*, 2003).

Immune genes in schizophrenia—Three independent groups found immune response pathway genes to be over-represented in postmortem schizophrenia DLPFC (Arion *et al*, 2007; Shao and Vawter, 2008; Saetre *et al*, 2007). The proteins encoded by genes for IFITM3 and serpin peptidase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 3 (SERPINA3) are involved in immune responses and are stimulated by cytokines (Andreu *et al*, 2006; Baker *et al*, 2007). Increases in mRNA for IFITM3 and SERPINA3 were also found in two schizophrenia cohorts (Saetre *et al*, 2007), in BA 9 (from the University of Pittsburgh Brain Bank), in BA 8 and BA 9 (from the Harvard and Stanley Brain Banks), and from the left side of the superior frontal gyrus (Arion *et al*, 2007).

IFITM3 and SERPINA3 are expressed in oligodendrocyte and endothelial cells, and their transcription is induced by the inflammatory cytokines TNF-α, IFN-α, and IFN-γ (Saetre *et al*, 2007). Increases in proinflammatory markers such as IL-6 are found in cerebrospinal fluid during exacerbations of psychotic disorders (Garver *et al*, 2003). The ability of

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haloperidol, risperidone, and clozapine to decrease proinflammatory markers in blood and microglia cell cultures (Kato *et al*, 2007; Moots *et al*, 1999; Song *et al*, 2000) suggests that decreases in inflammatory response might lessen schizophrenia. Lifetime antipsychotic level is associated with an increase in v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastomaderived oncogene homolog (ERBB2) transcripts in schizophrenia (Shao and Vawter, 2008), and ERBB2 protein in rat hippocampus (Chong *et al*, 2008). Neuregulin 1 (NRG1) and ERBB4 receptors are increased in schizophrenia in DLPFC (Chong *et al*, 2008). If either can cross the blood–brain barrier, the ERBB2 receptor antibody trastuzumab (Sastry and Sita Ratna, 2004) or the anti-inflammatory agent erythropoietin (EPO) (Ehrenreich *et al*, 2004, 2008) could be evaluated as treatments for schizophrenia by blocking excess immune activation (Hanson and Gottesman, 2005).

Gene changes as a basis for drug discovery—On the basis of an evaluation of gene expression in single stellate cells collected from the entorhinal cortex of normal controls and schizophrenic cases (Ginsberg *et al*, 2004; Hemby *et al*, 2002), and genomic profiling of dorsal root ganglia neurons collected by LCM (Luo *et al*, 1999), LCM was used to separate hippocampal dentate gyrus granule cell neurons from surrounding tissue (Figure 3) and profile gene expression with cDNA microarrays (Judd and Akiskal, 2003). These studies identified far more gene decreases in schizophrenia than expected by chance, and replicated 37% of these genes and 29% of the upregulated genes in a second cohort (Figure 8). Importantly, almost every gene that changed in both cohorts did so in the same direction. Functional groups of gene decreases were found to be highly significant by EASE analysis and survived Bonferroni correction for multiple comparisons. These functional groups included those involved in protein turnover (proteasome subunits and ubiquitin), similar to gene decreases found in the DLPFC (Vawter *et al*, 2001), and mitochondrial oxidative energy metabolism (including isocitrate, lactate, malate, NADH, and succinate dehydrogenases; complexes I–IV, cytochrome *C* oxidase, ATPase, and ATP synthase), as observed for mitochondrial gene decreases in adult rat brain of offspring born to vitamin Ddeficient dams (Eyles *et al*, 2007). These mRNA decreases were also found in the frontal cortex of schizophrenics (Middleton *et al*, 2002) and so too for many of the proteins they encode, and in different patient cohorts (Prabakaran *et al*, 2004; Whatley *et al*, 1996), and independent of patient medication status. In addition, chronic treatment of rats with clozapine failed to lower these genes in LCM-captured dentate granule neurons (Altar *et al*, 2005). In fact, MAD1 and other metabolic genes were unchanged or *increased* in brain by haloperidol treatment of monkeys or rodents (Middleton *et al*, 2002; Whatley *et al*, 1996).

Insulin/IGF-1 and muscarinic signaling—Many of the genes decreased in schizophrenia encode for mitochondria, carbohydrate metabolism, ubiquitin–proteosome pathways, ATP production, and hydrogen ion transport proteins and were also found by microarray studies to be decreased in skeletal muscle of diabetic rodents (Yechoor *et al*, 2002). Rome *et al* (2003) used microarrays to demonstrate the upregulation of many of these same genes by insulin in human skeletal muscle. These similar data sets indicated that brain insulin signaling might be deficient in schizophrenia. The insulin signaling pathway has been shown to be deficient in schizophrenia brain (Zhao *et al*, 2006) and olanzapine treatment elevates insulin mRNA in the frontal cortex of rats by 3.7-fold, the largest increase among all genes tested (Fatemi *et al*, 2006).

Using human SH-SY5Y neuroblastoma cell cultures, human insulin or insulin-like growth factor 1 (IGF-1) increased the expression of genes involved in mitochondrial function, the ubiquitin–proteosome pathway, glucose and energy metabolism, synaptic function, and hydrogen ion transport (Altar *et al*, 2008). These cells did not respond to IGF-2, BDNF, haloperidol, clozapine, or many other pharmacologically active compounds. These effects were consistent with the demonstration that insulin phosphorylated its receptor in the SH-

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SY5Y cells and, similar to IGF-1, increased the phosphorylation of the insulin-signaling mediators ERK1/2 and Akt. Using an algorithm similar to sections I and II of Table Box 1, 14 target genes were selected if their expression in dentate gyrus granule cells changed in the same direction in both schizophrenia cohorts, or were reported by others and found in at least one cohort to be altered in schizophrenia. The next level of priority was ascribed to disease signature genes that were closely related to a gene reported by others to change in the same direction in schizophrenia. Genes changed by insulin treatment of SH-SY5Y cells and in the opposite direction to that in schizophrenia brain were next most heavily weighted. Two control genes that did not change in schizophrenia or in response to insulin were chosen to provide normalization of the target genes (Figure 5 and Figure 9).

Responses of the 14 target genes to insulin, first identified with microarrays, were verified in the MPHTS^{SM} assay and by quantitative RT-PCR. With insulin serving as a positive control, 1940 pharmacologically defined compounds were screened for their mimicry of the insulin effect, and most were inactive. Interestingly, most of the active compounds were muscarinic agonists, and their effects on the schizophrenia genes were blocked by the muscarinic antagonists atropine and telenzepine (Altar *et al*, 2008). [^{3}H]Pirenzepine binding to muscarinic receptors is lower within dentate gyrus and CA1, CA2, and CA3 neurons, and M4 expression is decreased in schizophrenic hippocampus (Scarr *et al*, 2007). These decreases are consistent with the ability of the M1/M4 muscarinic agonist xanomeline to improve the positive and negative symptoms of schizophrenia (Bymaster *et al*, 2002). These findings indicate that schizophrenics, or a sub-population of them, may have an insulin or IGF-1 signaling deficit in the brain. Increases in muscarinic or insulin/IGF-1 signaling may normalize genomic alterations in schizophrenia and better address its root causes.

BPD—Individuals with BPD present initially with either a depressive or manic episode. The diagnosis of BPD type I follows a distinct period of mood change (elevated, expansive, or irritable) for at least a week, or shorter if hospitalization is required (DSM-IV criterion A). The manic episode can involve at least three or more items from the following (DSM-IV criterion B): inflated self-esteem or grandiosity, decreased sleep, more talkative or pressured speech, racing thoughts, distractibility, increased goaldirected activity, and excessive involvement in pleasurable activities. A hypomanic state, or BPD, type II, requires a shorter duration and severity of manic symptoms, and a depressive episode to have occurred (see Major Depressive Disorder below, for criteria). Diagnosis of BPD may require several years as a manic episode may not occur for several years after presentation. The prevalence of these DSM cases in the US National Epidemiological Catchment Area database is about 1.3%; however, subthreshold bipolar spectrum cases are about 5% (Judd and Akiskal, 2003). The course of bipolar illness can be quite severe, including a 10-fold increase in the risk of suicide above the general population, and a 20-fold increase for patients not treated with lithium (Tong and Jahr, 1994). The brevity of the following section and the short list of genes reported for BPD in Table 2 reflect the smaller number of confirmatory studies for genes and targets identified in BPD.

Gene changes in bipolar brain—The 14-3-3 family protein YWHAQ is decreased in the prefrontal cortex (Elashoff *et al*, 2007; Vawter *et al*, 2006a) and hippocampus (Konradi *et al*, 2004) of bipolar cases. YWHAZ is decreased in bipolar DLPFC, as determined by a reanalysis of other studies (Elashoff *et al*, 2007), and in suicide subjects, in BA 46 (Klempan *et al*, 2007). YWHAE is decreased in cortex–suicide age-matched pairs (Sibille *et al*, 2004) and increased by lithium (McQuillin *et al*, 2007). There have, however, been negative reports of 14-3-3 family member changes in postmortem tissue (Dean *et al*, 2007; Wong *et al*, 2005).

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The relevance of 14-3-3 proteins to BPD may also lie in their chaperone activity to enhance τ phosphorylation by glycogen synthase kinase 3 (GSK-3β) (Agarwal-Mawal *et al*, 2003). The phosphorylation of the N-terminal serine 9 site of GSK-3β inhibits its activity, as induced by lithium, leading to neuroprotection (Bachmann *et al*, 2005). This is consistent with pharmaceutical industry efforts to develop selective GSK-3β inhibitors that mimic lithium. τ and GSK-3β copurify with YWHAZ, and the association of phosphorylated GSK-3β with 14-3-3 *in vitro* prevents cell death (Mwangi *et al*, 2006; Henshall *et al*, 2002; Wang *et al*, 2007b). 14-3-3 family members also bind to the proapoptotic Bcl-2-associated death protein (BAD) to decrease cell death signaling (Henshall *et al*, 2002; Wang *et al*, 2007b; Shinoda *et al*, 2003).

Targets and drugs identified—The 14-3-3 gene expression studies in BPD reveal decreases in YWHA-Q, -Z, and -E. It is interesting to speculate that the liability of SSRI drugs to precipitate a manic episode may result from their ability to induce YWHAZ and YWHAE, which has been reported for fluoxetine (Cecconi *et al*, 2007). Control of 14-3-3 proteins could strengthen effects of serotonin, GABA, and acetylcholine function in the nervous system to provide an alternative treatment for BPD. Deficiencies in GABA gene expression have been reported by several groups in the brains of suicide cases, BPD, and major depressive disorder (MDD) (Klempan *et al*, 2007; Sequeira and Turecki, 2006; Sequeira *et al*, 2007).

Niculescu and colleagues (Le-Niculescu *et al*, 2007; Ogden *et al*, 2004) have used convergent functional genomics (CFG) in mouse models to rank genes that are in close (10 cM) proximity to bipolar risk candidate genes. The CFG paradigm cross-matches comprehensive animal model microarray gene expression data with human genetic linkage data, human postmortem gene expression data, and biological roles data. This Bayesian way of reducing uncertainty produces a short list of high-probability candidate genes, pathways, and mechanisms for complex genetic disorders, such as neuropsychiatric disorder (Bertsch *et al*, 2005).

The genes recently updated for BPD by the CFG approach include lysophospholipase I (LYPLA I), myocyte enhancer factor 2C (MEF2C), calcium/calmodulin-dependent protein kinase kinase 2,β (CAMKK2), cyclin-dependent kinase 5, regulatory subunit 1 (p35) (CDK5R1), tachykinin, precursor 1 (TAC1), proenkephalin (PENK), protein phosphatase 1, regulatory (inhibitor) subunit 1B, DA- and cAMP-regulated phosphoprotein (DARPP-32), T-box, brain, 1 (TBR1), cholecystokinin (CCK), myelin-associated oligodendrocytic basic protein (MOBP), and GLUL (Le-Niculescu *et al*, 2007). The CFG approach has been applied to a DPB (D-box-binding protein) knockout mouse model with features resembling BPD. The model was designed with information regarding blood-based biomarkers, QTL for behavioral changes, postmortem studies, and pharmacological and environmental stressors that induced gene expression changes (Le-Niculescu *et al*, 2007, 2008). The small number of samples used in these experiments, and the statistical likelihood that some disease- and drug-signature gene overlaps (Figure 1) is chance levels, requires that CFG associations be replicated.

Other gene changes between bipolar disease and drug paradigms include FGF2, for which increases were also reported in BPD (Shao and Vawter, 2008; Nakatani *et al*, 2006) and increased and decreased in subgroups of cocaine abusers (Lehrmann *et al*, 2003a, b). HINT1, summarized in the schizophrenia section, was decreased in a reanalysis of BPD microarray studies (Elashoff *et al*, 2007). Aldehyde dehydrogenase family 7 member A1 was increased in the DLPFC of schizophrenia and BPD in protein (Pennington *et al*, 2007) and mRNA measures (Shao and Vawter, 2008). Treatment of rats with lithium or valproate also increased hippocampal BAG-1 expression and BAG-1 protein (Zhou *et al*, 2005).

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GAD1 is decreased in the extracts of whole hippocampus (Konradi *et al*, 2004) but to a greater degree in stratum pyramidale and oriens of BPD cases, similar to GAD2 in stratum oriens (Benes *et al*, 2007).

Major depressive disorder—MDD is characterized by abnormal mood symptoms that occur during the same 2-week period that results in clinically significant impairment. Five or more of the following criteria from DSM-IV must be met: depressed mood, diminished pleasure, decreased concentration, fatigue, feelings of worthlessness, weight or appetite disturbance, sleep disturbance, psychomotor disturbance, and recurrent thoughts of dying. Individuals with major depressive mood disorder will not have manic, mixed, or hypomanic episodes; however, if these occur and are not associated with antidepressant treatment, then a diagnosis of BPD is given. The risk of suicide is also high for individuals with severe MDD, but lithium (Guzzetta *et al*, 2007) and antidepressant treatments reduce but certainly do not eliminate this risk (Healy and Whitaker, 2003; Mann *et al*, 2005). Suicide rates can approach 20% among severely depressed men in some countries and are generally 3–5 times higher in men than women. Understanding the pathophysiology of depressive disorders and providing better treatments will improve mental health and quality of life for the largest number of individuals with psychiatric disease.

Gene changes in depressive disorder brain (Table 2)—The enzyme GLUL is enriched in glia cells compared to neurons, where it converts glutamate to glutamine (ATP + $_{L}$-glutamate + NH_3 ⇔ ADP + orthophosphate + $_{L}$-glutamine) (Figure 10). GLUL is decreased in MDD (Choudary *et al*, 2005), BPD and schizophrenia (Shao and Vawter, 2008), and BPD and MDD (Vawter *et al*, 2006). GLUL expression is decreased by valproate administration (Bosetti *et al*, 2005) and is downregulated in 75% of MDD–suicide pairs (Sibille *et al*, 2004), in schizophrenics who died from suicide (Kim *et al*, 2007), and in suicide cases from a Montreal cohort (Klempan *et al*, 2007).

Another potential pathway for intervention in glutamate–glutamine metabolism is the shuttling of these compounds between astroglial end feet and neuronal processes (Figure 10) (Magistretti *et al*, 1999). The high-affinity glutamate transporter SLC1A2 (also known as EAAT2 or GLT) is brain enriched relative to peripheral organs (Berger and Hediger, 2006). Similar to GLUL, SLC1A2 is enriched in glial cells relative to neurons (Rothstein, 2003). It is decreased within the DLPFC in BPD and SZ (Shao and Vawter, 2008), in MDD (Choudary *et al*, 2005), in MDD–suicide cases (Klempan *et al*, 2007) and in 68% of MDD–suicide age-matched control pairs (Sibille *et al*, 2004). A note of caution has been raised regarding the specific isoform of importance because both EAAT2a and EAAT2b are alternatively spliced exons for the same SLC1A2 gene, and one study (Lauriat *et al*, 2006) failed to find changes in EAAT2, EAAT3, EAAT3b, or EAAT3 in DLPFC and visual cortex of schizophrenic cases. EAAT2a is the predominant isoform in human brain (Lauriat *et al*, 2006). The ability of the NMDA antagonist riluzole (Frizzo *et al*, 2004) to upregulate EAAT2 gene and protein expression, and the antidepressant effects of riluzole in treatment-resistant patients (Sanacora *et al*, 2007; Zarate *et al*, 2005), is consistent with the EAAT2 decrease in MDD and suicide. The approval of riluzole for amyotrophic lateral sclerosis (Rothstein, 2003) may provide an opportunity to identify antidepressant effects in these patients as well. An experimental drug that has shown efficacy in treating schizophrenia, LY2140023, is an oral prodrug for LY404039, a metabotropic GluR2/3 receptor agonist (Patil *et al*, 2007). Similar to β-lactam antibiotics (Rothstein *et al*, 2005), LY404039 increases astrocyte glutamate uptake, thus providing another compound with which to stimulate glutamate uptake to test this approach for treating MDD.

Another gene related to glutamatergic function is alanine-glyoxylate aminotransferase 2-like 1 (AGXT2L1), which participates in glutamate catabolism by transferring ammonia groups

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through alanine during glutamate–glutamine shuttling (Figure 10). A transaminase, AGXT2L1 promotes the conversion of L-alanine + glyoxylate ⇒ pyruvate + glycine, and participates in the metabolism of L-glutamate and 3-phosphonopyruvate ⇔ 2-amino-3-phosphonopropanoate + 2-oxoglutarate. This is consistent with its putative mitochondrial localization and with potential involvements in enzymatic amino-acid catabolism of glutamate, alanine, arginine, histidine, glutamine, and proline. AGXT2L1 is upregulated in BPD and schizophrenia (Shao and Vawter, 2008; Ryan *et al*, 2006) and downregulated in suicide completers (Kim *et al*, 2007) and in 72% of MDD–suicide cases when compared with age-matched controls (Sibille *et al*, 2004). Individuals with high AGXT2L1 appear to be at a higher risk of developing a psychiatric disorder, based on a highly significant odds ratio (Shao and Vawter, 2008). AGXT2L1 was the most upregulated gene in a genome-wide expression study of lithium administration to mice (McQuillin *et al*, 2007). Among schizophrenic cases, AGXT2L1 was decreased between the suicide completers vs the non-suicide group (Kim *et al*, 2007). AGXT2L1 expression was decreased in atypical neuroleptic-treated patients with schizophrenia when compared with controls (Shao and Vawter, 2008). AGXT2L1 may withstand independent validation as a novel target of lithium and neuroleptics, thus providing greater relevance to its decreases in depression and suicide, and increases in BPD and schizophrenia.

In summary (Figure 10), SLC1A2 and GLUL have defined roles in glutamate neurotransmission and decreases are associated with MDD. AGXT2L1 has a putative role in glutamate–glutamine conversion and alanine-lactate shuttling between neurons and glia, and is decreased in MDD and suicide. Recent successes in treating depression (Zarate *et al*, 2005, 2006; Sanacora *et al*, 2007), schizophrenia (Patil *et al*, 2007), and BPD (Mathew *et al*, 2008) with drugs that augment several forms of glutamate neurotransmission increase interest in these genes and glutamatergic pathways. SLC1A2, GLUL, and AGXT2L1 may have an interactive effect on increasing the risk for MDD, BPD, or schizophrenia by altering the processing of glutamate by astrocytes. *In vivo* imaging of the anterior cingulate cortex has shown lower glutamine concentrations in non-medicated adolescents with BPD (Moore *et al*, 2007) and these results are consistent with abnormalities within glial cells of the anterior cingulate cortex in BPD (Vawter *et al*, 2000). However, increases in anterior cingulate cortex glutamine levels may not be sufficient to treat mood disorders. The antiepileptic drug, topiramate, raises glutamine levels in the anterior cingulate cortex in healthy men (Moore *et al*, 2006), but large clinical trials failed to show that it is an effective treatment for BPD (Goodnick, 2006; Johannessen Landmark, 2008). Instead, augmentation of other aspects of glutamate function compromised by deficient glutamate metabolism in astrocytes or neurons (Figure 10) may be therapeutic for MDD. NMDA antagonism by riluzole and ketamine may increase AMPA signaling relative to NMDA signaling to treat depression (Maeng *et al*, 2008), whereas direct mGlu2 receptor agonism may treat psychosis (Patil *et al*, 2007).

FGF—FGF family members have been found to be dysregulated in multiple studies of MDD, including genes for FGF1, acidic; FGF2, basic; keratinocyte growth factor (FGF7); and receptors FGFR1, FGFR2, and FGFR3 (Sibille *et al*, 2004; Evans *et al*, 2004; Aston *et al*, 2005; Tochigi *et al*,). Interestingly, two studies reveal higher FGFR1 mRNA expression and reduced FGF2 mRNA in MDD hippocampus and PFC (Tochigi *et al*, 2007; Gaughran *et al*, 2006). These findings suggest decreased FGF synthesis, and possible compensatory increases in its receptor subtypes. Olanzapine is approved for mood stabilization in BPD and upregulates FGF3 and downregulates FGFR2 when administered to mice (Fatemi *et al*, 2006). These changes are in line with the FGF downregulation–receptor upregulation hypothesis we propose for mood disorder. It will be of interest to profile other drugs approved for bipolar mood stabilization, such as Abilify, valproate, carbamazepine, and lithium, to see whether there is a common effect on the FGF ligand and receptor system as

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described for olanzapine. Lithium also produces reciprocal changes by decreasing FGF3 and increasing its receptor, FGFR3, in neuronal cells (Yazlovitskaya *et al*, 2006). ECS, a model for electroshock therapy, increases cortical and hippocampal mRNA for the angiogenic factors VEGF, FGF, and neuropilin. These angiogenic factor increases are unexpected candidate targets for treating mood disorders including MDD and could underlie the antidepressant effects of ECT and exercise (Altar *et al*, 2005; Newton *et al*, 2003). Importantly, intracranial infusions of FGF reduce depressive-like behaviors in the forced swim test (Turner *et al*, 2006, 2008), and increase memory (Ishihara *et al*, 1992) and neurogenesis (Cheng *et al*, 2002; Matsuoka *et al*, 2003).

The FGF system offers additional therapeutic alternatives to the monoamine-based approaches that dominate treatment strategies for mood disorders (Turner *et al*, 2006). Peptide mimetics derived from the neural cell adhesion molecule (NCAM1) can also bind to and activate FGFR (Anderson *et al*, 2005; Berezin and Bock, 2004), (Figure 11). These NCAM1 mimetics enhance social memory retention (Secher *et al*, 2006), increase neurite outgrowth (Li *et al*, 2005), act as an antidepressant in animal models of depression (Turner *et al*, 2008), and are currently in clinical trials for AD (Anand *et al*, 2007). Similar to FGF, expression of the heterologous FGFR ligand, NCAM1, is decreased in MDD (Tochigi *et al*, 2007) and MDD–suicide pairs (Sibille *et al*, 2004), whereas proteolytically cleaved fragments of NCAM1 are elevated in BPD and schizophrenia (Vawter, 2000). Pathways that play roles in mood disorders, including MAP kinase and PI3/Akt signaling pathways, are modified by FGF-induced transcription factors that activate cadherin gene transcription or bind cadherin molecules (El-Hariry *et al*, 2001a, b; Boscher and Mege, 2008; Murakami *et al*, 2008). These interactions are summarized in Figure 11.

Other genes—G-protein-coupled receptor 37 endothelin receptor type B-like (GPR37) and G-protein-coupled receptor, family C, group 5, member B (GPRC5B) are decreased in the superior temporal gyrus in MDD (Aston *et al*, 2005) and in the prefrontal cortex of most MDD–suicide cases *vs* age-matched controls (Sibille *et al*, 2004). These orphan GPCRs will require more investigation to determine endogenous ligands, functions, and possibly small molecules that interact with them.

Two microarray studies (Aston *et al*, 2005; Tochigi *et al*, 2007) independently found two genes, DIM1 dimethyl-adenosine transferase 1-like (DIMT1L) and PRP19/PSO4 pre-mRNA processing factor 19 homolog (PRPF19) to be decreased in MDD. Comparisons across microarray MDD studies showed NTRK2 (TrkB, the high-affinity receptor for BDNF and NT-3) to be decreased in more than one study, as did SSAT, CTSB, COPA, and PTP4A2 (Sequeira *et al*, 2006, 2007; Sequeira and Turecki, 2006; Choudary *et al*, 2005; Aston *et al*, 2005; Tochigi *et al*, 2007; Evans *et al*, 2003). Decreases in BDNF and TrkB have received additional confirmation by Dwivedi *et al* (2003, 2005).

Aquaporin 4 (AQP4), the predominant water-selective membrane channel in brain, is concentrated in astrocytic end feet and its expression is increased in the DLPFC of MDD and BPD cases (Iwamoto *et al*, 2005; Tochigi *et al*, 2007). Interestingly, AQP4 is reduced in rat brain by lithium (McQuillin *et al*, 2007) and valproate (Bosetti *et al*, 2005), and altered in 80% of MDD–suicide pairs (Sibille *et al*, 2004).

Gene effects of electroconvulsive seizure—ECT alleviates depression in the majority of drug-resistant patients with MDD or BPD. To discover targets for rapid pharmacological treatment of MDD, microarray-based genomic profiling studies of neocortex and hippocampus (Altar *et al*, 2004; Newton *et al*, 2003) have measured gene changes in rodent brain following ECS, a model of ECT. These findings have been compared with gene changes in the CNS in other antidepressant models, such as exercise

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(Ang and Gomez-Pinilla, 2007; Molteni *et al*, 2002), or the administration of antidepressant drugs to animals or cultured neurons.

Many more genes were found to change to ECS in the hippocampus than in the neocortex, and a single ECS produced more changes than did 10 daily ECS administrations (Figure 12). Gene changes were ranked by an algorithm similar to that in Table Box 1 according to their statistical and biological significance, identification by other laboratories, and whether they changed in one or both tissues, or after one or both durations of ECS administration (Altar *et al*, 2004). This analysis identified 54 of the 88 mRNA or protein changes to ECS reported by others in rodent brain, and identified 101 novel genes.

In total, 30 genes that increased in response to ECS participate in biochemical pathways which have already been associated with the antidepressant efficacy (Altar *et al*, 2004; Newton *et al*, 2003) (Figure 13). These include genes involved in neurogenesis, neuritogenesis, neuropeptide synthesis, neurotransmitter signaling, angiogenesis, and arachidonic acid metabolism. Immediate-early genes increased by acute and chronic ECS include c-fos, jun, jun-B, Krox20, Nurr1, TGFβ-inducible ERG (TIEG), the activity regulated cytoskeleton-associated growth factor (arc), NGF, and the activity and neurotransmitter-induced early gene proteins 4 (ania-4) and 3 (ania-3).

BDNF and TrkB message and protein are decreased in the postmortem neocortex and hippocampus of suicide cases (Dwivedi *et al*, 2003, 2005; Karege *et al*, 2005). Interestingly, decreases in BDNF and NT-3 were found in drug-free but not medicated suicides, suggesting upregulation of these factors by psychotropic agents (Karege *et al*, 2005). Consistent with this proposal, ECS elevated 17 genes along the BDNF/TrkB-stimulated MAP kinase pathway and its convergent, glutamate-coupled PKC pathway, and four additional genes of the ERK1,2-linked arachidonic acid pathway (Figure 13). ECS increases BDNF mRNA (Duman *et al*, 1997; Nibuya *et al*, 1995; Zetterstrom *et al*, 1998) and protein (Altar *et al*, 2003b) in rat neocortex and hippocampus. Several antidepressants or candidate drugs, including the glutamate release-augmenting drug riluzole (Katoh-Semba *et al*, 2002) and the AMPAkines LY 392098 or CX614 (Lauterborn *et al*, 2000), enhance BDNF expression and granule precursor cell proliferation in hippocampus. BDNF mRNA and protein, and TrkB mRNA, are increased in rat pups after 13 days of treatment with the SSRI escitalopram (Kozisek *et al*, 2008). The importance of TrkB phosphorylation and signaling (Saarelainen *et al*, 2000), and Grb-2 and RAS activation for antidepressant action, may occur through the enhanced intracellular signaling through MAP kinase, RAS, and cAMP by antidepressant therapies (Mathew *et al*, 2008; Duman *et al*, 1997; Skolnick *et al*, 2001; Altar, 1999). These may represent novel targets for depression that avoid the nonspecific effects of ECT (Table Box 3).

ECS is also a potent inducer of hippocampal neurogenesis (Hellsten *et al*, 2002; Madsen *et al*, 2000) and ECS increases the sprouting of serotonin fibers (Madhav *et al*, 2001) and mossy fibers from dentate granule neurons (Chen *et al*, 2001). Twelve of the genes augmented by ECS might contribute to these actions. These include BDNF, TrkB, HES-1, neuritin, glypican, protein tyrosine phosphatase, Vesl/homer and its isoform, CREM, and neurofilament genes. Neuritin, similar to glypican, encodes a neuronal protein that promotes the outgrowth and branching of neuritic processes in hippocampal and cortical cells, where it is highly expressed (Naeve *et al*, 1997). Interestingly, dorsal raphe serotonin cell bodies are diminished by over 50% (Arango *et al*, 2001), and serotonergic axon densities are decreased by 24% in the deeper layers of DLPFC of suicide patients with MDD (Austin *et al*, 2002). The ability of BDNF (Mamounas *et al*, 1995) and ECS (Madhav *et al*, 2001) to augment serotonergic axon fiber densities in intact or fiber-lesioned rats is consistent with an antidepressant effect of BDNF (Siuciak *et al*, 1997) and may be mediated through a BDNF-

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and glutamate-stimulated neuritin/Vesl/homer pathway (Altar, 1999; Naeve *et al*, 1997; Sato *et al*, 2001).

Eleven genes, including BDNF, Cox-2, Vesl/homer, VGF, NGF-inducible clone C, are upregulated in rat hippocampus by exercise (Molteni *et al*, 2002; Russo-Neustadt *et al*, 1999; Tong *et al*, 2001) and by ECS in the hippocampus (Altar *et al*, 2004). These overlapping genes are of particular interest because of the moderate antidepressant and neurogenic effects of exercise (Craft *et al*, 2007; Labbe *et al*, 1988). VGF expression is decreased in the hippocampus of mice rendered helpless by forced swimming (Thakker-Varia *et al*, 2007). Similar to BDNF (Siuciak *et al*, 1997), intracranial infusions to mice of a synthetic VGF-derived peptide (Hunsberger *et al*, 2007) or VGF itself (Thakker-Varia *et al*, 2007) produce antidepressant-like responses in the forced swim and tail suspension tests. Mice lacking the VGF gene show a pro-depressant effect and an attenuated antidepressant-like response to exercise (Hunsberger *et al*, 2007). These findings, and the ability of imipramine as well as ECS to elevate arc and VGF protein levels in the hippocampus (Thakker-Varia *et al*, 2007), suggest that enhanced VGF expression by BDNF signaling could be a mechanism for antidepressant agents. BDNF induction of hippocampal VGF, arc, c-fos, EGR1, and other genes through MAP kinase (Alder *et al*, 2003) is consistent with this proposal.

FUTURE RESEARCH DIRECTIONS

The present review has identified genes and pathways as candidates for understanding psychiatric and neurodegenerative diseases, as well as possible targets for drug discovery. We reported findings from human brain tissues that were replicated in multiple cohorts of cases and by multiple laboratories, or were confirmed by protein-based immunohistochemistry, ELISA, or other detection methods, or by more precise RNA detection methods such as RT-PCR. A far larger number of genes can be found in the literature that did not meet these criteria, and thus were not described here.

As exemplified by glutamate–glutamine shuttling, mitochondrial metabolic processes, FGF and neurotrophic factor signaling, responses to ECS, and pathways mediated by several G-protein-coupled receptors, multiple genes could be assembled into pathways. The aggregate functions of these pathways appear to be compromised in CNS disease. Some gene clusters seemed to be implicated in diverse diseases (eg, neurotransmitters, 14-3-3 proteins, glutamate binding and transport, metabolic dysfunction), but in general, each disease appears to involve distinct genes and pathways. These findings represent a start to a more complete picture of dysregulation. As new findings emerge, it is likely that some findings reported here will fall by the wayside, whereas others will be reinforced.

These multi-gene and pathway targets provide a novel approach to identify compounds based on their ability to alter genes in a desired manner. This screening approach (Figure 1) was demonstrated for schizophrenia and depression, where proven therapeutic approaches (muscarinic agonism and ECS, respectively) alter genes in neuron cultures or rodent brain in a reciprocal manner to their change in diseased brain, and in a similar manner to other therapeutic approaches, such as exercise for depression. It will be of interest to see whether new treatment modalities, such as transcranial magnetic stimulation for depression, or metabotropic GluR agonists for schizophrenia or anxiety, alter a subset of the same genes as do established therapies, or reciprocally alter disease signature genes.

Compounds identified by multi-gene screening will still require preclinical proof of concept through the use of *in vivo* models. In schizophrenia, these may include existing models, such as pre-pulse inhibition of startle responses, PCP models of psychosis, and models of working memory and cognition. But compounds discovered through actions on novel drug

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targets will probably require novel models, such as mice with heterozygous deletions or overexpression of receptors implicated in the identified pathways. It will also be valuable to determine proof of concept in animal models, such as in mouse strains prone to depressive-like behaviors, for which a manipulation by ECS or administration of the newly implicated factor, VGF, produces antidepressant-like effects.

An alternative proof-of-concept approach is to determine whether genes identified in the brains of affected patients are altered in a reciprocal manner in the homologous brain area of animals exposed to candidate and therapeutic compounds. For example, the rapid antidepressant response to intravenous injections of the NMDA antagonist ketamine (Zarate *et al*, 2006; Berman *et al*, 2000) increases interest in the genes in mood disorders (Vawter *et al*, 2006; Aston *et al*, 2005) that may also be affected by ketamine administration (Lowe *et al*, 2007). Such ketamine-responsive genes may help identify rapidly acting antidepressants (Zarate *et al*, 2006; Mathew *et al*, 2008; Maeng *et al*, 2008). In either case, lead compounds will need to be evaluated with more traditional ADME/toxicology and PK/PD models to satisfy FDA requirements for clinical testing prior to phase I and II trials. Measures of target mRNA changes in brain can be combined with traditional measures, such as *in vivo* binding or enzyme changes, to answer traditional questions in drug development such as blood–brain barrier penetration, oral availability, and dosing ranges. This information can be used to maximize efficacy in new behavioral tests.

The era of CNS functional genomics is in its infancy, but is starting to provide a list of options for rational drug design that go beyond the re-derivatization of marketed drugs that have modest effects. The discoveries of drug candidates that treat AD, PD, depression, psychosis, and BPD through interactions with non-monoaminergic targets provide an exciting opportunity to reconcile their novel mechanisms with genes reported here, and with the gene effects of anti-Parkinsonian drugs, antidepressants, anti-psychotics, and mood stabilizers. In the interim, gene discovery may reinforce targets that are already being pursued, and help guide the selection of secondary models. Only by testing in humans will the validity of the CNS pharmacogenomic approach to drug discovery be determined.

Acknowledgments

Dr Vawter received funding from NIMH Biomarker Grant, NIMH Conte Center For Genetics of Depression, Pritzker Neuropsychiatric Disorders Research Consortium, and William Lion Penzner Foundation. Dr Ginsberg is supported by grants NS48447, AG14449, AG17617, and the Alzheimer's Association.

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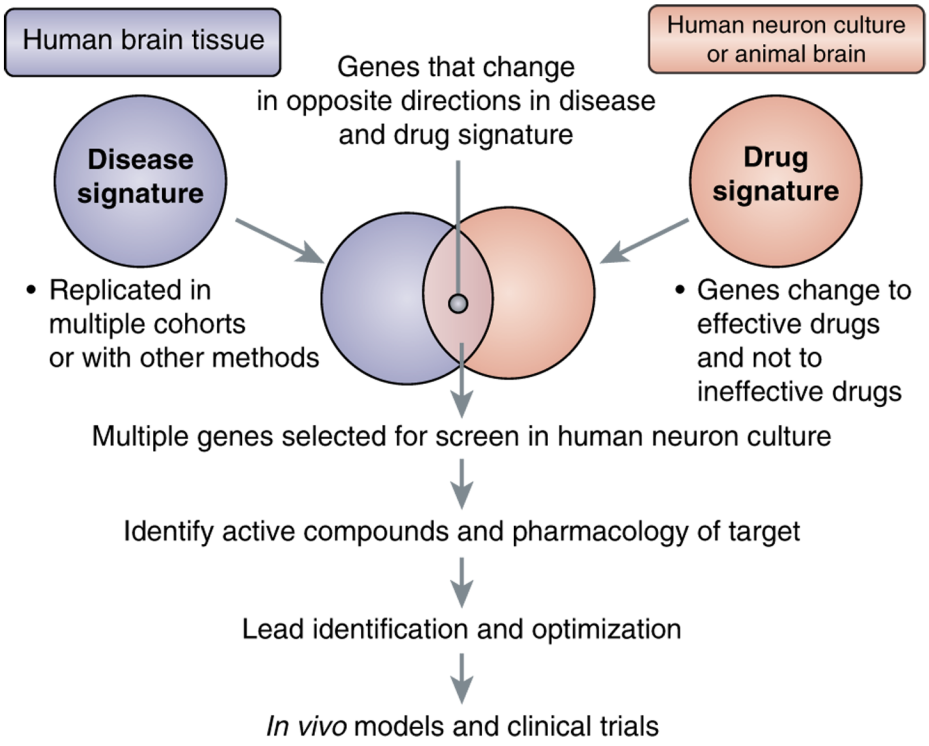

Figure 1.
Gene-based drug discovery strategies based on disease-specific (left) and drug-induced (right) mRNA expression changes. Information from these data, the literature, and other factors described in the text are used to select a smaller set of genes that change reciprocally between human brain and drug-exposed tissue, and are used for gene-based compound screening or identification of specific targets.

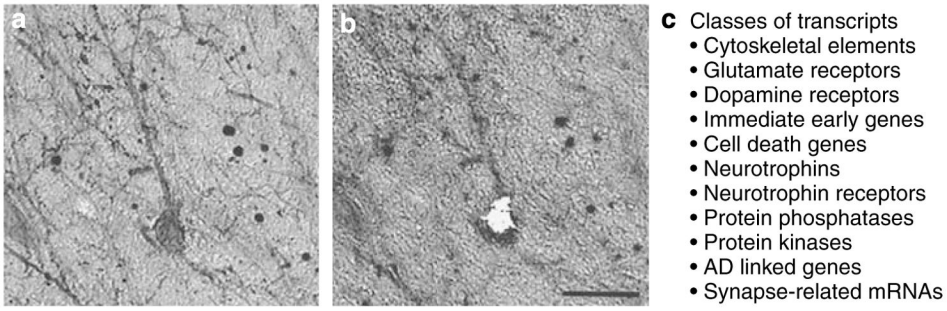

Figure 2.

Single-cell microaspiration and classes of transcripts on a custom-designed cDNA array. (a) A $p75^{NTR}$-immunoreactive neuron in the nucleus basalis of an AD patient is shown. (b) Same section as (a) following single-cell microaspiration. (c) Classes of transcripts analyzed in single-cell preparations using a custom-designed cDNA array platform (n = 576 genes). Scale bar: 50 μm. Adapted from Ginsberg *et al* (2000) with permission.

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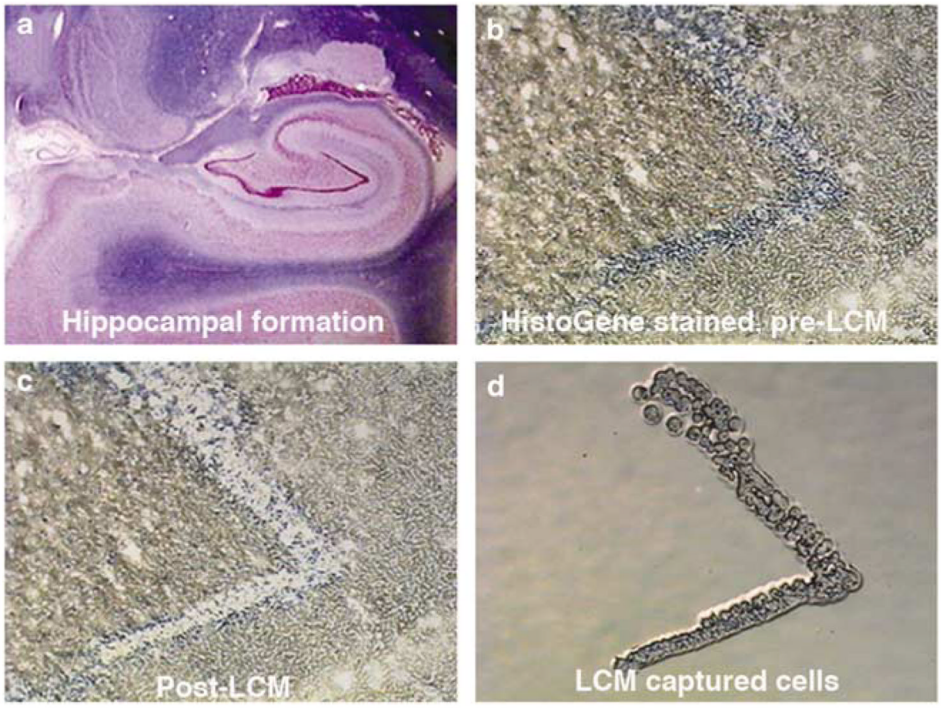

Figure 3.
LCM is used to collect dentate neurons from the human hippocampus (shown in lower power, top left) (a). The top right micrograph (b) is an enlargement that shows the blue-stained dentate neurons before LCM. In the lower right (d), the LCM captured neurons are isolated and gene expression is measured in cDNA produced from their amplified RNA. The 'hole' after LCM can be seen in the lower left micrograph (c).

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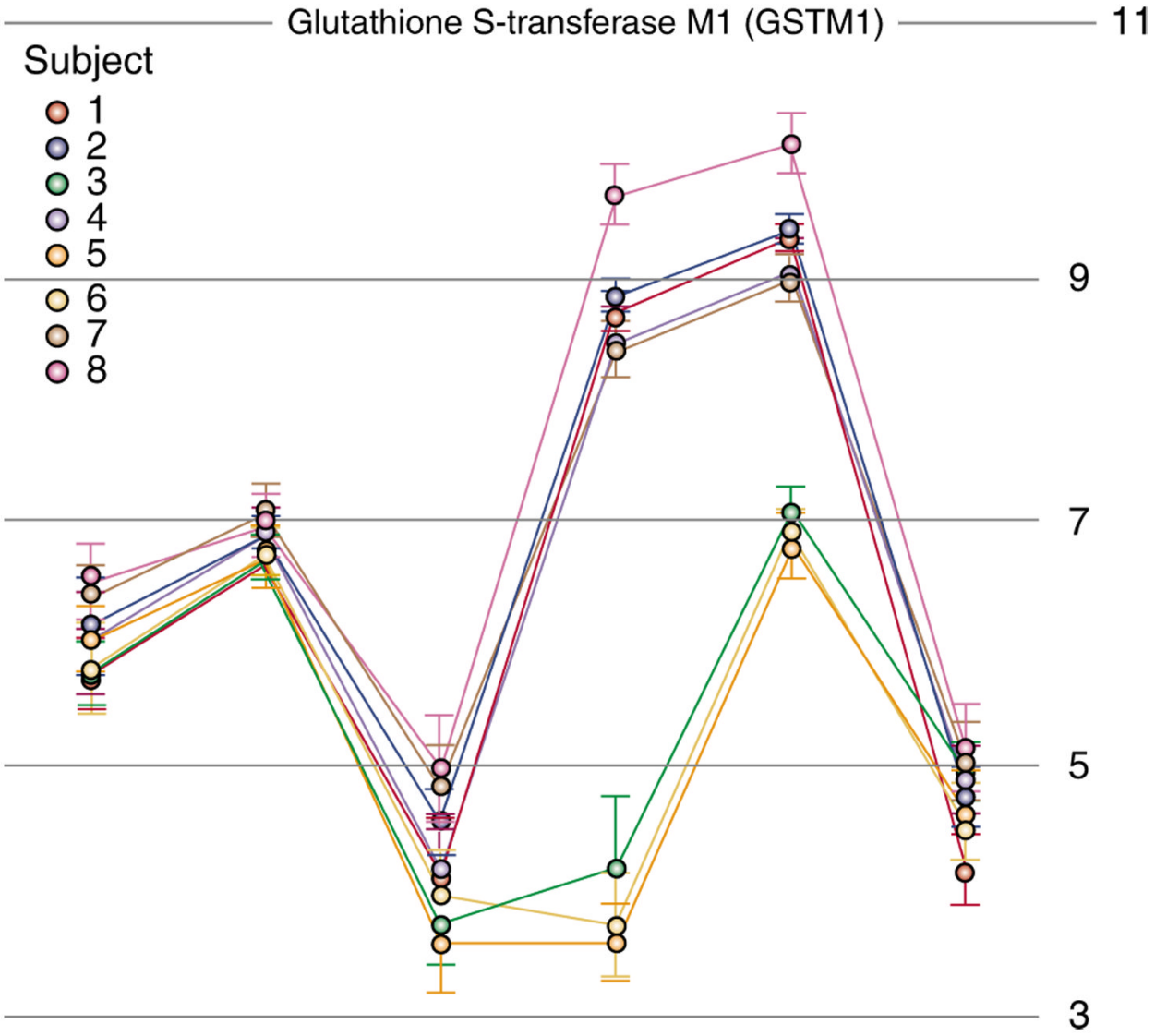

Figure 4.
Splicing variations in glutathione *S*-transferase M1 (GSTM1) between eight individuals identified by exon array. The X axis points represent six exons within this gene. The *Y* axis represents the relative abundance of their expression. Note that the 3′ (left) and 5′ (right) ends of the gene show similar expression levels among people, but the fourth and fifth exons of the GSTM1 gene vary in the expression of this transcript.

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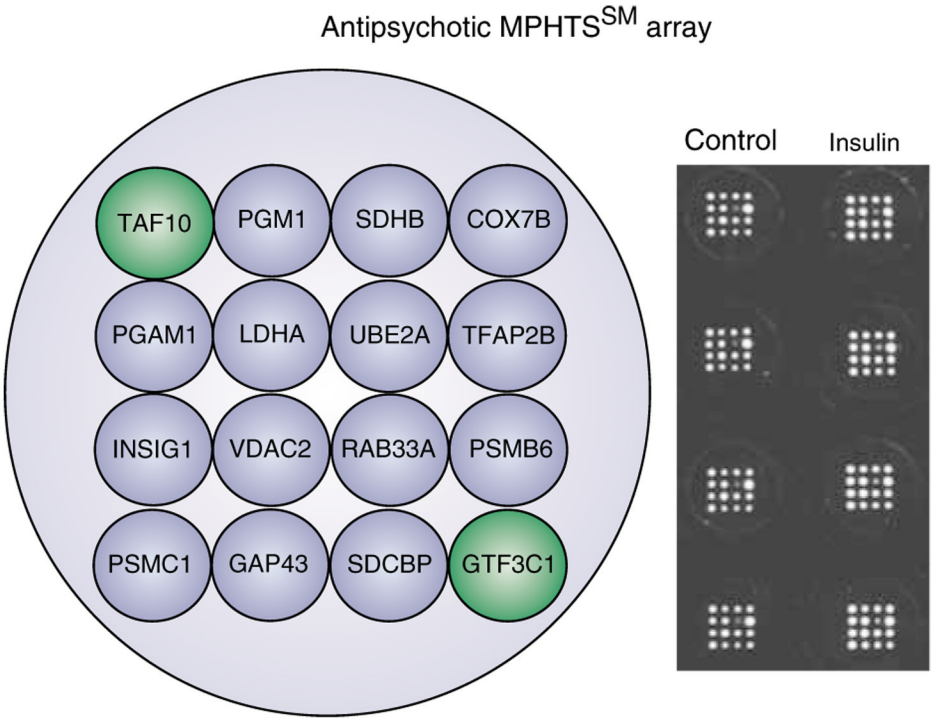

Figure 5.

Gene miniarray for antipsychotic drug discovery. Human neuroblastoma cells were cultured for 24 h in wells of a 96-well plate, in the presence of vehicle or 20 nM human insulin. RNA from the lysed cells was added to the MPHTS^{SM} plate, which contains the illustrated 4 × 4 array of spotted cDNA for the genes. Typical increases by insulin *vs* the vehicle (control) were observed in quadruplicate by changes in fluorescence for each gene relative to two control genes, TAF10 and GTF3C1.

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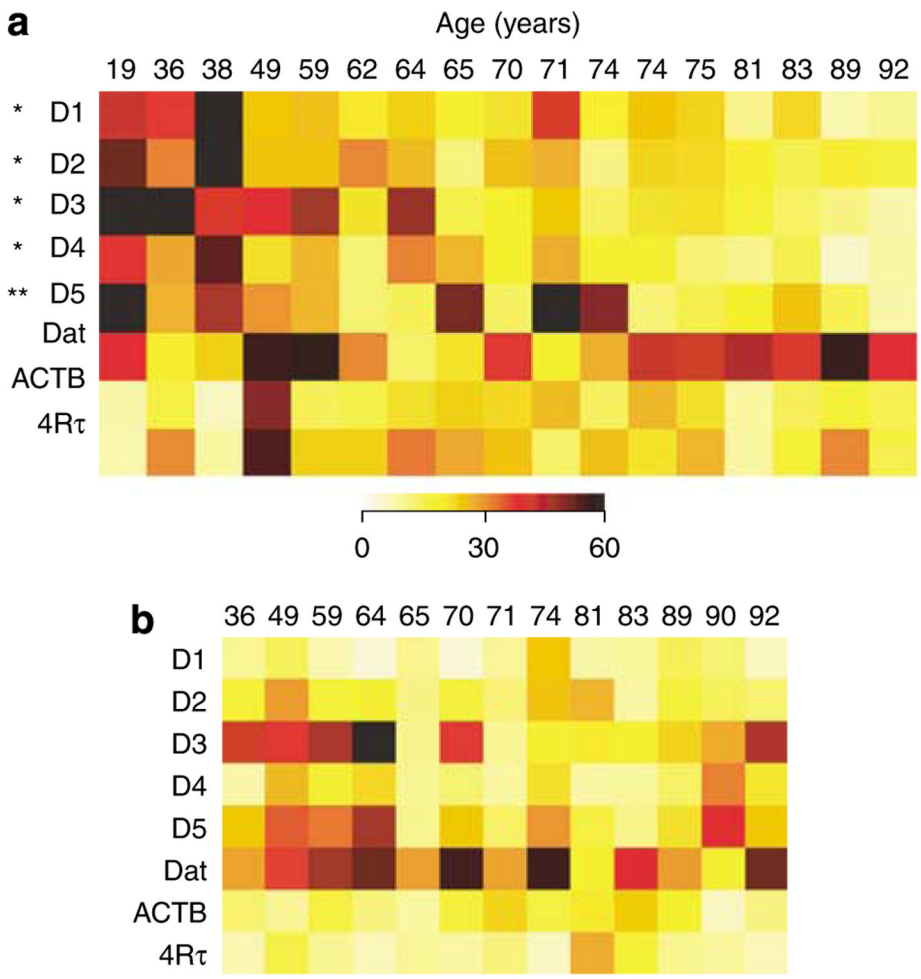

Figure 6.

Expression profiling in neurofibrillary tangles (NFTs) bearing CA1 pyramidal neurons in postmortem AD brain. (a) Dopamine (DA) receptors D1–D5 are significantly decreased (*p<0.001; *p<0.05) in AD NFT-bearing CA1 neurons as evidenced by representative custom cDNA arrays. (b) Downregulation of several synaptic-related markers (*p <0.01; including synaptotagmin, SYP, synapsin I, α-synculein, and β-synuclein) is presented in a representative custom cDNA arrays and a histogram. In contrast, the relative expression of 4Rτ did not vary across the cohort, although the ratio between 3R/4Rτ is altered in AD. Adapted from Galvin and Ginsberg (2005) and Ginsberg *et al* (2000) with permission.

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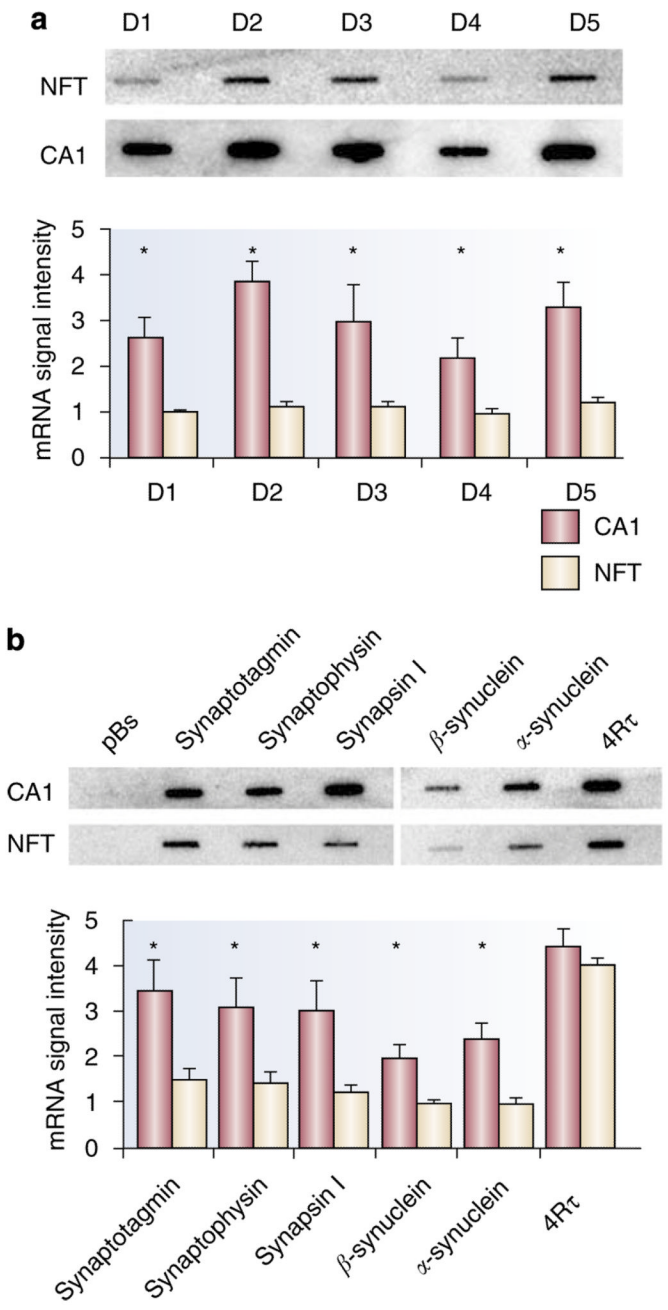

Figure 7.

D1–D5 receptor expression levels in normal aging. The color-coded heatmap illustrates differential regulation of each receptor within individual CA1 pyramidal neurons and entorhinal stellate cells across a normal aged cohort. (a) Relative expression levels of the DA receptors, dopamine transporter (DAT), β-actin (ACTB), and 4-repeat τ (4Rτ) within individual CA1 pyramidal neurons microaspirated from postmortem human brains (aged of 19–92 years) (Hemby *et al*, 2003). Significant downregulations of DRD1–DRD5 were found (*p<0.001. No differential regulation of DAT, ACTB, and 4Rτ was observed, indicating a relative specificity of age-related transcript decline. (b) In contrast to the observations in CA1 pyramidal neurons, no differential regulation of DA receptors was observed within

individual entorhinal cortex stellate cells. Adapted from Galvin and Ginsberg (2005) with permission.

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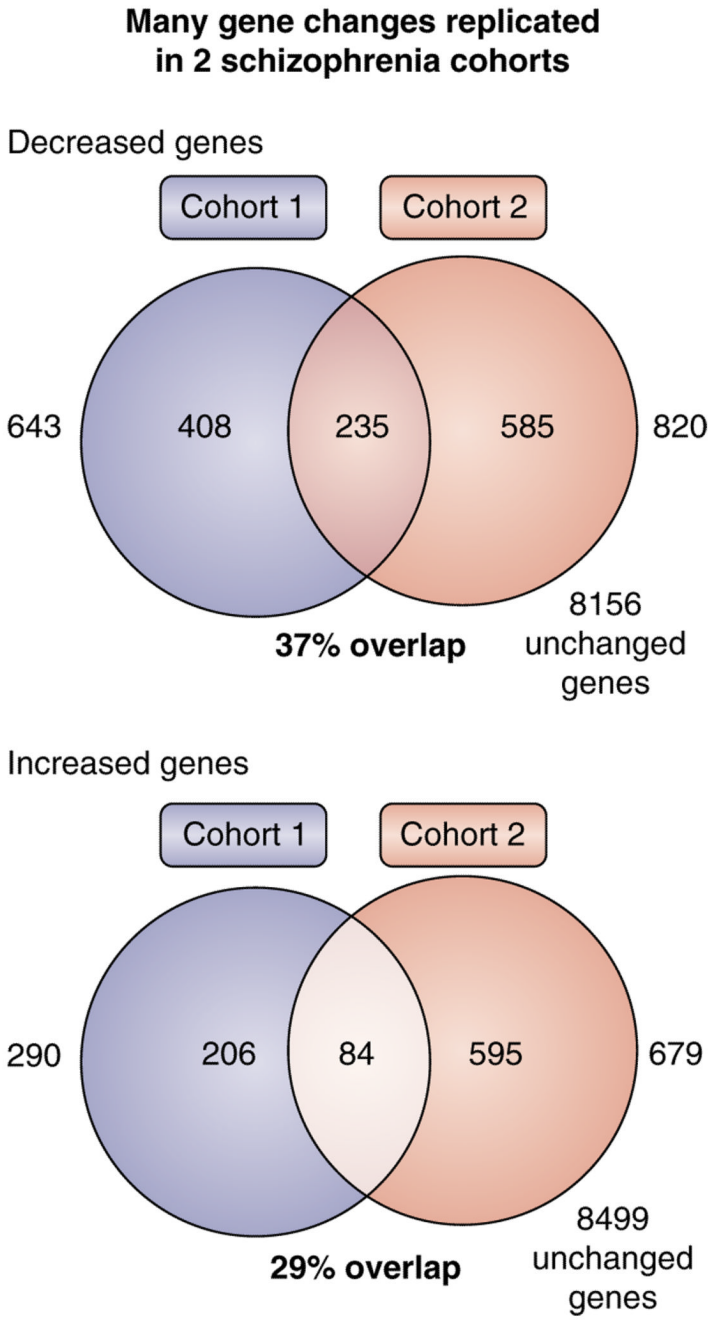

Figure 8.
Overlap in genes decreased (top) and increased (bottom) between two cohorts of schizophrenic cohorts and controls, $n = 9$ controls and 8 schizophrenics (cohort 1), and 15 controls and 14 schizophrenics (cohort 2). Over 95% of the genes that changed ($p<0.05$) in both cohorts changed in the same direction (Altar *et al*, 2005).

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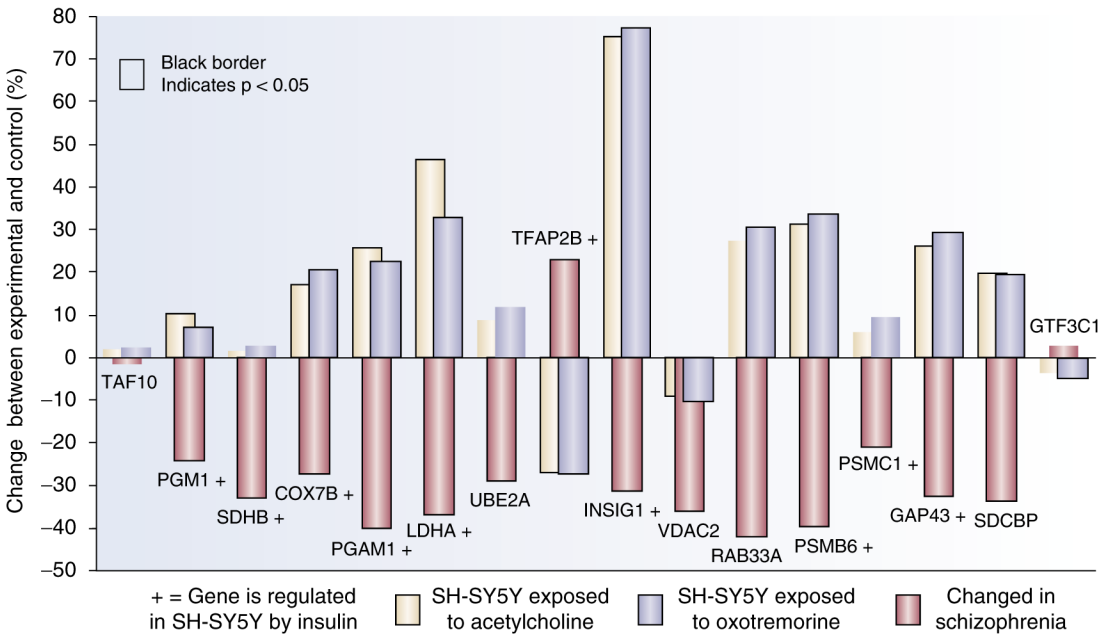

Figure 9.

Reversal of schizophrenia gene signature (red bars) (Altar *et al*, 2005) by muscarinic agonists, 25 μM acetylcholine (yellow bars) and 25 μM oxotremorine (blue bars; Altar *et al*, 2008). A '+' next to gene names signifies that it changed in response to insulin, and in a direction similar to the muscarinic agonists. Black border around bars indicates a significant change from vehicle control, $p<0.05$.

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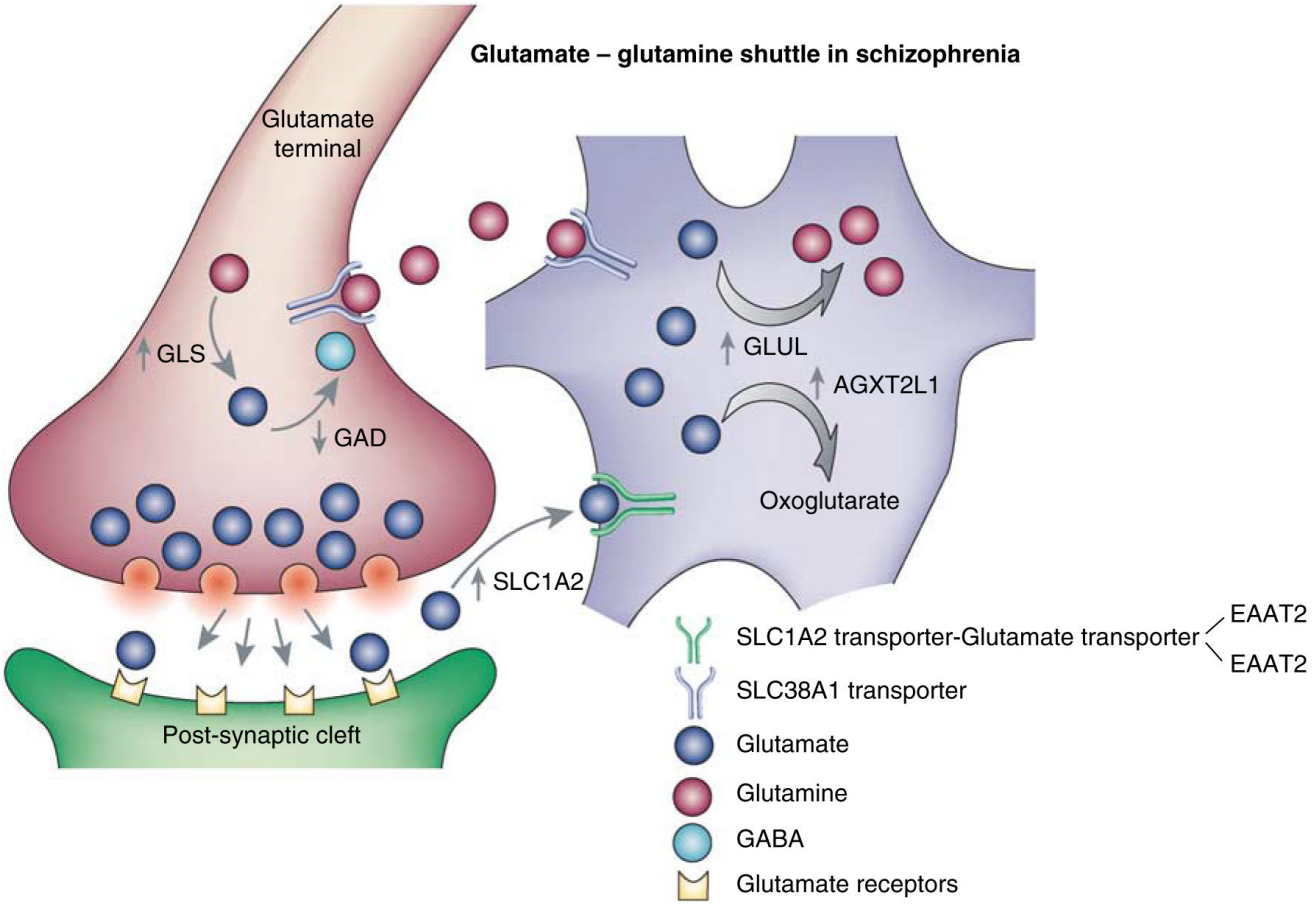

Figure 10.

Glutamate–glutamine shuttle between glutamatergic nerve terminals and astroglia in the CNS. Increased expression of GLUL and SLC1A2 genes in schizophrenia and bipolar disorder (Shao and Vawter, 2008; Vawter *et al*, 2006a; Choudary *et al*, 2005; Beasley *et al*, 2006) and increased expression of GLS (Bruneau *et al*, 2005; Gluck *et al*, 2002) have been reported in schizophrenia, whereas decreased SLC1A2 and AGXT2L1 mRNA and GLUL mRNA and protein levels in major depressive disorder in the anterior cingulate were reported (Shao and Vawter, 2008; Vawter *et al*, 2006a; Choudary *et al*, 2005; Beasley *et al*, 2006). These molecules contribute to the transport, synthesis, and recycling of glutamate after synaptic release (Magistretti *et al*, 1999). Glutamate does not cross the blood–brain barrier and its presence in the CNS is from the glia-derived precursor, glutamine. SLC1A2 (solute carrier family 1 (glial high-affinity glutamate transporter, member 2)), AGXT2L1 (alanine-glyoxylate aminotransferase 2-like 1), GLUL (glutamate-ammonia ligase (glutamine synthetase)), SLC38A1 (solute carrier family 38, member 1), and GLS (glutaminase) are involved in this process and, as shown by arrows, are mostly increased in schizophrenia.

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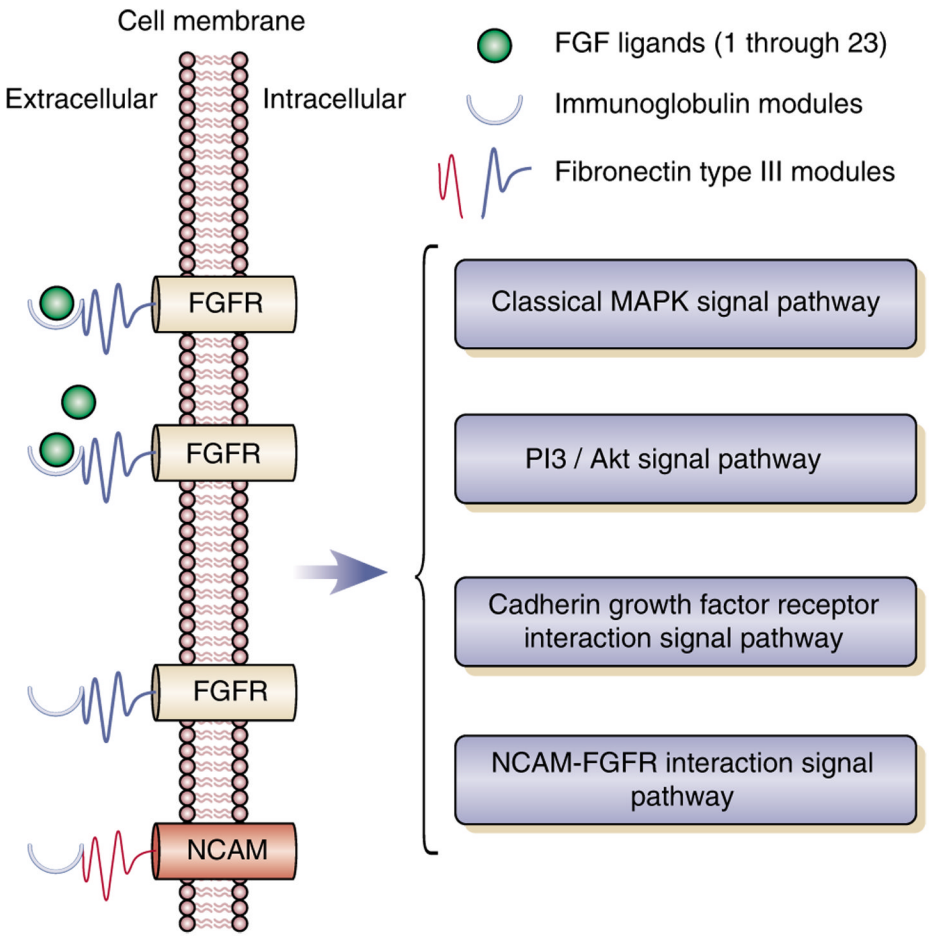

Figure 11.

Fibroblast growth factor (FGF) signaling through diverse pathways. In total, 23 FGF family member ligands bind to four different FGF receptors (FGFR 1, 2, 3, and 4) and activate downstream signaling through FGFR homodimerization (shown by FGFR and FGFR). FGF signaling promotes the transcription of genes involved in proliferation, energy, growth, survival, angiogenesis, neurite extension, and growth cone motility. These signaling pathways can also be triggered by neural cell adhesion molecule (NCAM1) binding to an FGFR1 or FGFR2–NCAM heterodimer (Doherty and Walsh, 1996; Christensen *et al*, 2006). Peptide mimetics are being tested for their NCAM-like binding to FGFR1 or 2. Such agonism is anticipated to treat cognitive deficits, schizophrenia, and AD (Secher *et al*, 2006; Klementiev *et al*, 2007).

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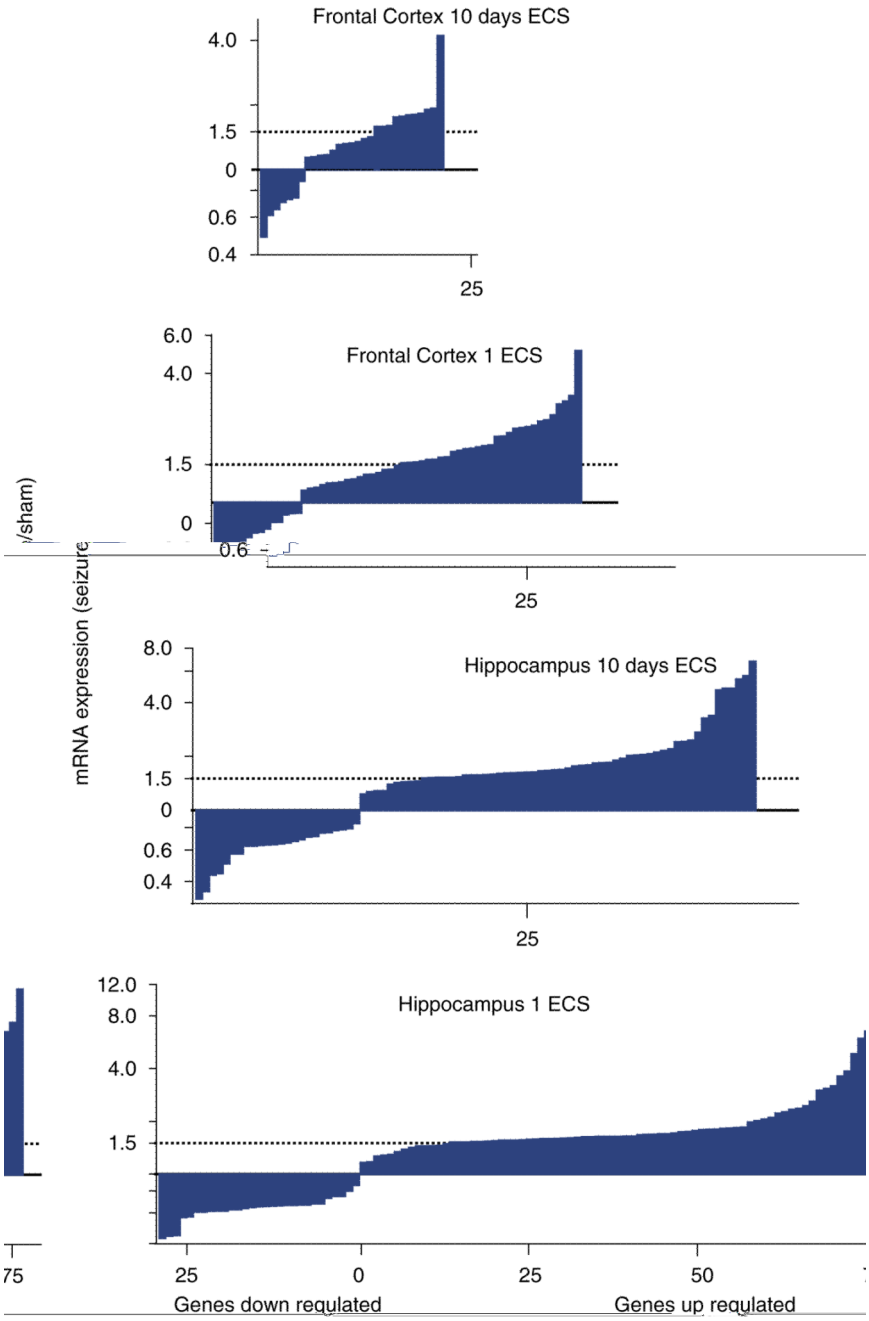

Figure 12.

Cumulative number (*X* axis) and fold change (*Y* axis) of gene changes in the frontal cortex and hippocampus of rats treated with acute or chronic ECS. For all genes that changed ($p < 0.05$) from sham seizure control rats, the ratio of the mean expression in the ECS group over the control group is cumulated over the magnitude of change. In each graph, decreases are plotted below the unity line and increases are plotted above the line. The *X* axes for all four graphs are aligned at '0 genes changed' and plotted at the same scale so that the width of the graph represents the number of genes that were significantly increased or decreased by ECS (Altar *et al*, 2004).

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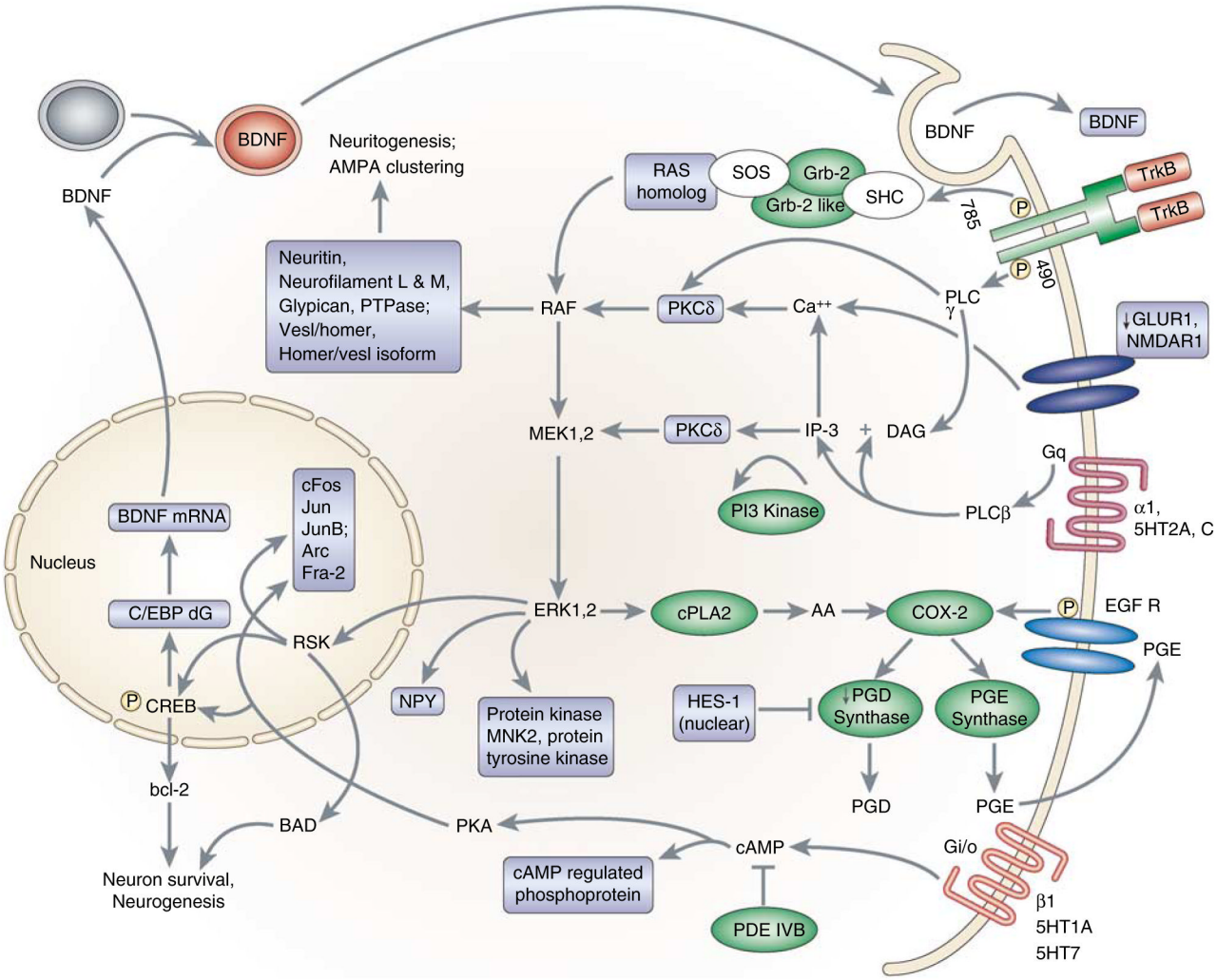

Figure 13.

Biochemical pathways implicated in the responses to ECS, based on statistically significant increases in gene expression (colored rectangle or oval labels) in the rodent hippocampus and/or frontal cortex following chronic ECS (Altar *et al*, 2005). GLUR1 and PGD synthase were decreased by ECS. Also reported by other groups in response to ECS, these genes included those within neurotrophic signaling pathways, including those for BDNF/MAP kinase, cAMP, and arachidonic acid signaling.

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Table 1

Genes changes in neurodegenerative diseases.

			REFERENCES	
Gene symbol	**Gene name**	**Statistically significant change**	**Initial publication**	**Confirmation**
Alzheimer's Disease				
DRD2	Dopamine receptor 2	Decreased	Joyce, 1993; Ryoo, 1994	Ginsberg, 2001
GRIA1	AMPA1 receptor	Decreased	Yasuda, 1995; Ikonomovic, 1995	Carter, 2004; Ginsberg, 2001
GRIA2	AMPA2 receptor	Decreased	Yasuda, 1995; Ikonomovic, 1995	Carter, 2004; Ginsberg, 2001
CRIN1	NMDA R1	Decreased	Yasuda, 1995	Ginsberg, 2001; Jacob, 2007
SLC1A1	Glutamate transporter EAAT3	Decreased	Ginsberg, 2001	Jacob, 2007; Westphalen, 2003
SYP	Synaptophysin	Decreased	Callahan, 1999	Gutala 2004 Ginsberg, 2001
SYT1	Synaptotagmin I	Decreased	Ginsberg, 2001	Gutala, 2004; Callahan, 1999
CHRNA7	α7 nicotinic receptor	Increased by ECS	Counts, 2007	Counts, 2004
3Rtau/4Rtau	Tau protein isoform	Increased ratio	Ginsberg, 2006	Ginsberg, 2006
APP	Amy**l**oid precursor protein	Present In SP	Ginsberg, 1999	Ginsberg, 2004
bax	bcl2-associated × protein	Present In SP	Ginsberg, 1999	Ginsberg, 2004
bcI-2	b-cell cl**l**/**l**ymphoma 2	Present In SP	Ginsberg, 1999	Ginsberg, 2004
GRIA1, GRIA2, GRIA4, and GRIK1	AMPA GluR subunits	Present in SP	Ginsberg, 1999	Ginsberg, 2004
GFAP	G**li**a**l** fibrilary protein	Present in SP	Ginsberg, 1999	Ginsberg, 2004
IL-1	**I**nter**l**eukin-1	Present in SP	Ginsberg, 1999	Ginsberg, 2004
AGER	Advanced glyucation end-products receptor	Present in SP	Ginsberg, 1999	Ginsberg, 2004
Alzheimer's disease/MCI				
TrkA	High affinity NGF receptor	Decreased	Ginsberg, 2001	Ginsberg, 2001; Boissiere, 1997
TrkB	High affinity BDNF, NT-3 receptor	Decreased	Ginsberg, 2001	Ginsberg, 2001
TrkC	High affinity NT-3, BDNF receptor	Deceased	Ginsberg, 2001	Ginsberg, 2001
Parkinson's disease				
UCH-L1	Ubiquitin C-terminal hydrolase L1	Decreased	Grunblatt, 2004	Liu, 2002; Choi, 2004
SKP1	S-phase kinase-associated protein 1A	Decreased	Grunblatt, 2004	Grunblatt, 2007
EGLN1	Ion transport	Decreased	Grunblatt, 2004	Grunblatt, 2007
Nurr-1	Orphan nuclear receptor	Increased by ECS	Newton, 2003; Altar, 2005	Chu, 2006; Eells., 2002
BDNF	Brain-derived neurotrophic factor	Increased by ECS	Newton, 2003; Altar, 2005	Hyman, 1991; Altar, 1994; 2003
TrkB	Tropomyosin receptor-related Kinase	Increased by ECS	Nibuya,1995; Altar, 2005	Hyman, 1991; Altar, 1994

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			REFERENCES	
Gene symbol	**Gene name**	**Statistically significant change**	**Initial publication**	**Confirmation**
gstm1	Glutathione-S-transferase	Increased by ECS	Altar, 2005	Hyman, 1991
S100β	S100 calcium binding protein	Increased by ECS	Newton, 2003	Altar, 2005
Normal aging				
PKC α, β, γ	Protein kinase α, β, γ subunits	Decreased	Lu, 2004	
CALM1	Calmodulin 1	Decreased	Lu, 2004	
Tau	tau protein	Decreased	Lu, 2004	
GIurR1	AMPA receptor subunit	Decreased	Lu, 2004	Ginsberg, 2007
EAAT2	Excitatory amino acid transporter 2	Decreased	Lu, 2004	Ginsberg, 2007
D1 through D5	Dopamine receptors 1 through 5	Decreased	Hemby, 2003	Ginsberg, 2004

ECS = **el**ectroconvulsive shock; SP = senile plaques.

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TABLE 2

Gene changes in psychiatric disease

			REFERENCES	
Gene symbol	**Gene name**	**Statistically significant change**	**Initial publication**	**Confirmation**
Schizophrenia				
UCHL1	Ubiquitin carboxyl-terminal esterase L1 (Ubiquitin thiolesterase)	Decreased	Vawter,2001	Middleton, 2002, Altar, 2005, RNA: Behan, 2008, protein
GAD1	Glutamic acid decarboxylase 1 (67kDa)	Decreased	Mirnics, 2000; Guidotti, 2000	Vawter, 2002; Straub, 2007; Addington, 2005; protein decrease, Hashimoto, 2005, 2008; Hashimoto and Lewis, 2006, Veldic 2008; but increased mRNA, Hakak, 2001 and protein, Knable, 2002 reported. Paulson *et al* 2003, rat cortex protein after MK-801
IFITM3	Interferon induced transmenbrane protein 3	Increased	Arion, 2007; Saetre, 2007	Shao, 2008; Saetre, 2007; Arion, 2007 for other assays
SERPINA3	Serpin peptidase inhibitor, clade A(alpha-1 antiproteinase, antitrypsin), member 3	Increased	Arion, 2007; Saetre, 2007	Shao, 2008; Saetre, 2007; Arion, 2007 for other assays
MAG	Myelin associatedd glycoprotein	Decreased	Hakak,2001	Aston, 2004
MDH1	Malate dehydrogenase 1	Decreased	Hakak,2001 only increse reported; Vawter, 2002	Shao, 2008, Vawter, 2004, Iwamoto, 2005, Middleton, 2002; Altar, 2005, MAD2
RGS4	Regulator of g protein signaling 4	Decreased	Mirnics, 2001	Arion, 2007; ISH In 3 cortical areas, Mirnics, 2001
SYN2	Synapsin II	Decreased	Mirnics, 2000; Vawter, 2002	Mirnics, 2000, ISH; Lee, 2005; Saviouk, 2007
TF	Transferrin	Decreased	Hakak, 2001	Saetre, 2007, Arion, 2007, RNA; Behan, 2008 Prabakaran, 2004, protein
HINT1	Histidine triad nucleotide binding protein 1	Decreased	Vawter, 2001, 2002, 2004, ISH.	Liu, In Press; Barbier, 2007, HINT 1 in basket neurons decreased in schizophrenia
GLUL	Glutamate-ammonia ligase		Shao, 2008	Kim, 2007; Burbaeva, 2003; Prabakaran, 2004
GLS	Glutaminase, phosphate activated	Increased	Gluck, 2002	Bruneau, 2005
YWHAH	14-3-3 protein eta	Decreased	Vawter, 2001	Iwamoto, 2005; Middleton, 2005; Wong, 2003, genetic association with schizophrenia; DiGeorge syndrome.

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			REFERENCES	
Gene symbol	**Gene name**	**Statistically significant change**	**Initial publication**	**Confirmation**
YWHAB	14-3-3 protein beta	Decreased	Middleton, 2005	Iwamoto, 2005; Dean, 2007 haloperdiol increases
YWHAE	14-3-3 protein epsilon	Decreased	Middleton, 2005	Novikova, 2006; Middleton, 2005; Iwamoto, 2005
YWHAG	14-3-3 protein eta	Decreased	Middleton, 2005	Iwamoto, 2005
YWHAQ	14-3-3 protein theta	Decreased	Middleton, 2005	Iwamoto, 2005
YWHAZ	14-3-3 protein zeta	Decreased	Vawter, 2001	Middleton, 2005; Iwamoto, 2005
BDNF	Brain-derived neurotrophic factor	Decreased	Weickert, 2003 protein and mRNA	Hashimoto, 2005, Weickert, 2005
NTRK2	Tropomyosin receptor related kinase 2, TrkB	Decreased	Hashimoto, 2005; Weickert, 2005	Hashimoto, 2005, repicated TrkB & BDNF decrease in a second cohort
GRIA2	Glutamate receptor, ionotrophic, AMPA 2	Decreased	Mirnics, 2000; Vawter, 2002	Beveridge, 2008
ALDH7A1	Aldehyde dehydrogenase family 7 member A1	Increased	Pennington, 2007	Shao, 2008
VDAC1	Voltage-dependent anion channel 1	Decreased	Hakak, 2001, the only increase reported	Iwamoto, 2005, Altar, 2005, RNA; Behan, 2008, Prabakaran, 2004, protein
GAP43	Growth-associated protein 43	Decreased	Altar, 2005; Hakak, 2001, the only increase reported	Behan, 2008, Prabakaran, 2004, Knable, 2002, Chambers, 2005, protein
CALB1	Calbindin 1	Decreased	Altar, 2005	Knable, 2002, protein
ENO1	Alpha enolase	Decreased	Prabakaran, 2004 (protein)	Behan, 2008
BASP1	Brain acid soluble protein 1	Increased	Prabakaran, 2004 (protein)	Behan, 2008
PRDX2	Peroxiredoxin-2	Decreased	Prabakaran, 2004 (protein)	Behan, 2008
ALDOC	Fructose biphosphate aldolase C	Decreased	Prabakaran, 2004 (protein)	Behan, 2008
DPYSL2	Dihydropyrimidinase related protein 2	Decreased	Prabakaran, 2004 (protein)	Behan, 2008
CHRM4	Muscarinic 4 receptor	Decreased	Scarr 2007; Reversal of schizophrenia signature with muscarinic agonists	M1 agonism: antlpsychotlc properties in schizophrenia (Bymaster, 2002), Alzheimer's (Bodick, 1997), and in monkey (Andersen, 2003) & rat (Stanhope, 2001) models.
Bipolar disease				
FGF2	Fibroblast growth factor 2	Increased	Shao, 2008; Nakatani, 2006	
HINT1	Histidine triad nucleotide-binding protein 1	Decreased	Elashoff, 2007, meta-analysis	Wang, 2007; Barbier, 2007. KO mouse mania
ALDH7A1	Aldehyde dehydrogenase family 7 member A1	Increased	Shao, 2008	Pennington, 2007

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			REFERENCES	
Gene symbol	**Gene name**	**Statistically significant change**	**Initial publication**	**Confirmation**
YWHAQ	14-3-3 protein theta	Decreased	Konradi, 2004	Elashoff, 2007; Vawter, 2006
YWHAZ	14-3-3 protein zeta	Decreased	Elashoff, 2007	Klempan, 2007
YWHAE	14-3-3 protein epsilon	Decreased	Sibille, 2004, suicide	McQuillin, 2007, increased by lithium
AGXT2L1	Alanine-glyoxylate aminotransferase 2-like 1	Increased	Shao, 2008	Kim, 2007, Sibille, 2004, decreased in suicides
AQP4	Aquaporin 4	Increased	Sibille, 2004, suicide	Reduced in rat brain by lithium McQuillin, 2007 and valporate Bosetti, 2005
Major depressive disorder				
GLUL	Glutamate-ammonia ligase	Decreased	Choudary, 2005	Vawter, 2006; Shao, 2008; Sibille, 2004, Kim, 2007 & Klempan, 2007, decreased in suicide
SLC1A2/EAA T2	Astrocyte high-affinity glutamate trasporter	Decreased	Choudary, 2005	Shao, 2008; Sibille, 2004 & Klempan, 2007, decreased in Suicide; Frizzo, 2004, mRNA and protein incsed by riluzole
AGXT2L1	Alanine-glyoxylate aminotransferase 2-like 1	Decreased	Kim, 2007 and Sibille, 2004, suicide	McQuillin, 2007. lithium increases in rat brain
FGF1	Fibroblast growth factor protein 1 (acidic)	Decreased	Evans, 2004; Sibille, 2004	Aston, 2005; Tochigi, 2007
FGF2	Fibroblast growth factor protein 2 (basic)	Decreased	Evans, 2004; Sibille, 2004	Aston, 2005; Gaughran, 2008; Tochigi, 2007
FGFR1	Fibroblast growth factor receptor 1	Increased	Gaughran, 2006; Tochigi, 2007	Evans, 2004; Sibille, 2004; Aston, 2005; Tochigi, 2007
FGFR2	Fibroblast growth factor receptor 2	Increased	Evans, 2004; Sibille, 2004	Aston, 2005; Tochigi, 2007
FGFR3	Fibroblast growth factor receptor 3	Increased	Yazlovitskaya, 2006, lithium increases	Evans, 2004; Sibille, 2004; Aston, 2005; Tochigi, 2007
NCAM1	Neural cell adhesion molecule	Decreased	Tochigi, 2007	Sibille, 2004. MDD suicide pairs; Vawter, 2000, cleavage products increased in BPD
GPR37	G protein-coupled receptor 37 endothelin receptor type B-like	Decreased	Aston, 2005	Sibille, 2004. MDD suicide pairs.
GPRC5B	G proein-coupled receptor, family C, group 5, member B	Decreased	Aston, 2005	Sibille, 2004, MDD suicide pairs.
DIMT1L	DIM1 dimethyladenosine transferase 1-like	Decreased	Aston, 2005	Tochigi, 2007
PRPF19	PRP19/PSO4 pre-mRNA processing factor 19 homolog	Decreased	Aston, 2005	Tochigi, 2007
NTRK2	Tropomyosin receptor related kinase 2, TrkB	Decreased	Dwivedi, 2003; trkB and BDNF decreases in suicide	Antidepressant effect of ICV BDNF in rats

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			REFERENCES	
Gene symbol	**Gene name**	**Statistically significant change**	**Initial publication**	**Confirmation**
				(Siuciak, 1997); rat ECS increases BDNF protein (Altar, 2001) and mRNA (Altar, 2005; Duman, 1997; others). TrkB activation required for antidepressant-induced behavioral effects (Saarelainen, 2003)
AQP4	aquaporin4	Increased	Iwamoto, 2005; Tochigi, 2007	McQuillin,2007, Sibillie, 2004; Bosetti, 2005 (decreased by lithium and valproate)

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Table Box 1

Alogrithm to Prioritize Genes that Change in Disease and Response to Treatment

I. *Statistical considerations based on gene changes in multiple studies*

A gene fold change from control and the significance (*p*-value) and reproducibility of that change are assigned a 'statistical score':

Fold change score=the fold change if gene increases, and 1/% of control value if the gene decreases

p-Value score = $-\log_{10}$(*p*-value)

Reproducibility score = × 2 for I replication of co-directional, statistically significant gene change, × 4 for 2 replications

Statistical score = (fold change score × *p*-value score) reproducibility score

II. *Biological considerations based on human and animal studies of the gene that changed*

A. Gene change is in the same direction of mRNA of protein change in human disease

B. Gene change is in the same direction of mRNA of protein change in animal model of the disease

C. Gene or its protein product change in the opposite direction in animals by treatments for that disorder, or when manipulated in transgenic animal, it models part of the disease pathology

D. Gene is part of a biochemical pathway associated with human disease or its treatment

E. Gene's human homolog is in a chromosomal hot spot for disease as identified by linkage analysis

Biological score = A (10)+B (8)+C (8)+D (5)+E (2); maximum = 33

The values in parentheses are summed for each criterion that is true for A–E

III. *Pharmacological considerations*

The score is based on how 'drugable' the target is, and whether it has received support in the past as a drug target

A. Gene changes in disease or in response to therapeutic agents are found to be under the control of a drugable target (ie, receptor or enzyme antagonist)

B. For antagonist approach, knockout of the target mimics the desired gene changes, or overexpression mimics the disease phenotype. Gene changes are reversed in knockout model by effective drugs.

C. Gene or its protein product is changed by effective treatments for that disorder or when manipulated in transgenic animal, predictably affect disease pathology.

Pharmacological score = A (10)+B (9)+C (8); maximum = 27

The values in parentheses are summed for each criterion that is true for A–C

Algorithm score = Statistical score+biological score+pharmacological score

Example of a robust gene:

A gene is doubled in bipolar disorder with a *p*-value of 10^{-5}, and its increase is replicated in two other studies. If the gene fulfills all biological and pharmacological criteria, its algorithm score will be about the maximum, or (2 × 5) × 4+33+27 = 100

This algorithm includes features that have been used to identify genes associated with schizophrenia (Altar *et al*, 2008) and the therapeutic response to ECT (Altar *et al*, 2005). The statistical, experimental, biological, and pharmacological considerations are quantified and summed to prioritize the significance of each gene as a target for CNS drug discovery.

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Table Box 2

Protein Changes Concordant in Two Studies of Schizophrenia (Prabakaran *et al*, 2004; Behan *et al*, 2008) Compared to Results for the Same Gene Found in Microarray Studies

	Direction of fold change in schizophrenia		
Symbol	**Protein by Behan *et al* (2008) and Prabakaran *et al* (2004)**		**mRNA change in the order of the cited reference(s)**
ATP5A1[a]	↑	↓↓↓↑	Altar *et al* (2005); Middleton *et al* (2002); Arion *et al* (2007); Iwamoto and Kato (2006)
ATP5B[a]	↓	↓↓	Iwamoto and Kato (2006)
ATP5H[a]	↑	↓	Iwamoto and Kato (2006)
ATP6V1E1	↓	↓	Altar *et al* (2005)
ATP6V1B[a]	↑	↑	Hakak *et al* (2001)
ALDH1A1	↓	↓	Iwamoto and Kato (2006)
CNP	↓	↓	Hakak *et al* (2001)
EHD3	↓	↓	Saetre *et al* (2007)
ESD	↓	↓	Altar *et al* (2005)
ENO2	↓	↑	Vawter *et al* (2002a)
ENO1	↓	↓	Paulson *et al* (2003)
DNM1	↑	↑	Vawter *et al* (2002a)
GAP43	↓	↑↓	Altar *et al* (2005); Knable *et al* (2002); Hakak *et al* (2001)
GSN	↓	↓	Saetre *et al* (2007)
GLUL	↓	↓	Shao and Vawter (2008)
HSPA1A	↓	↓	Arion *et al* (2007)
HSPA2	↓	↓	Hakak *et al* (2001)
HSPA8	↓	↓	Arion *et al* (2007)
LHPP	↓	↓↑	Saetre *et al* (2007)
MDH1	↓	↑↓↓↓↓	Vawter *et al* (2002a, 2004b); Middleton *et al* (2002); Hakak *et al* (2001); Iwamoto *et al* (2005); Shao and Vawter (2008)
NDUFV2	↑	↓	Iwamoto *et al* (2005)
NSF	↑	↓	Mirnics *et al* (2000); Vawter *et al* (2002a)
PDHA1	↓	↓	Iwamoto and Kato (2006)
PDHB	↑	↓↓↓	Altar *et al* (2005); Iwamoto and Kato (2006)
PGAM	↓	↓	Altar *et al* (2005)
TF	↓	↓↓↓	Hakak *et al* (2001); Arion *et al* (2007); Saetre *et al* (2007)
UCHL1	↓/↑	↓↓↓	Altar *et al* (2005); Middleton *et al* (2002); Vawter *et al* (2001)
UQCRC1	↓/↑	↓	Iwamoto *et al* (2005)
VDAC1	↓	↓↑↓	Altar *et al* (2005); Hakak *et al* (2001); Iwamoto *et al* (2005)
YARS	↓	↓	Arion *et al* (2007)

[a] A finding by Behan *et al* (2008), without a result from Prabakaran *et al* (2004). BASP1 was increased, and PRDX2, ALDOC, and DPYSL2 were decreased in both studies, but without confirmatory findings.

Arrows indicate direction of significant fold change in each listed study, in the same order as the listed studies. Gray-filled boxes indicate concordant mRNA expression and protein changes.

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Table Box 3

Potential Antidepressant Pathways and Associated Gene Targets Based on the Predominant Pathways Summarized in Figure 13

Biochemical pathway	Gene target	Class and example	Target and drug implicated in treating depression?
Neurogenesis	↑ BDNF	SSRI (fluoxetine, escitalopram)	Yes
	↑ TrkB	MAOI (tranylcypramine)	Yes
	↑ VGF	AMPAkines (CX 516;LY 392098); riluzole	Yes
	↑ PKC	mGLUR agonists (LY 404039)	Yes
Excitatory amino acid pathways	↑ NMDA activity	Glycine and analogs	?
	mGLUR1	Glycine uptake inhibitors	?
	↑ Vesl/homer	Ampakines (LY 392098); riluzole	Yes
		mGLUR antagonists (LY 367385)	Yes
Cyclic AMP	↑ cAMP-regulated phosphoprotein	5-HT1A agonists (buspirone, gepirone)	Yes
		5-HT7 agonists (5-CT)	No
		β1 agonists (prenalterol)	No
	↓ PDE IVB	Selective PDE inhibitors based on rolipram, vipocetine	Yes
Phospholipase/MAP kinase	↑ PI3 kinase	LY 294002 (antagonist)	?
		PD 98059 (antagonist)	?
Prostaglandins/arachidonic acid	↑ cPLA2	Melitin	Yes
	↑ COX2	Valproate; celecoxib, rofecoxib (antagonists)	Yes
Vascular genesis and remodeling	↑ VEGF	VEGF, bFGF	Yes

Validation by drugs used to treat depression is indicated in the last column.

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APPENDIX A

Representative a brain banks for neurodegenerative and psychiatric disease cases

Brain bank	Website
Center for Neurodegenerative Disease Research, University of Pennsylvania	http://www.med.upenn.edu/cndr/
Cognitive Neurology and Abheimer's Disease Center at Northwestern University	http://www.brain.northwestern.edu/mdad/neuropathology.html
Columbia University	http:://www.nybb.hs.columbia.edu/
Harvard Brain Tissue Resource Center	http://www.brainbank.mclean.org/
Multiple Sclerosis Brain Bank	http://www.msbrainbank.org.au/
New South Wales Brain Bank	http://www.florey.edu.au/support/australian-brain-bank-network/
NINDS Web site	http://www.ninds.nih.gov/funding/research/parkinsonsweb/brainbanks.htm
Push Alzheimer's Disease Center	http://www.rush.edu/rumc/page-R12388.html
Stanley Medical Research Institute	http://www.stanleyresearch.org/dnn/BrainResearchCollection/tabid/83/Default.aspx
University of Miami Brain Endowment Bank	http://brainbank.med.miami.edu/
University of Pittsburgh Alzheimer Disease Research Center Brain Bank	

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APPENDIX B

Commercial and non-commercial microarray platforms and software sources used to conduct microarray gene expression studies

Platform vendor	**Websites for information on products**
Affymetrix	www.affymetrix.com/index.affx
Exon Hit	www.exonhit.com/index.php?page=1
Agilent	www.chem.agilent.com494 www.chem.agilent.com/Scripts/PCol.asp?lPage=494
Illumina	www.illumina.com/
Applied Microarray (formerly Codelink GE Amersham)	www.appliedmicroarrays.com/Human.html
Roche Nimblegen	www.nimblegen.com/products/exp/index.html
Software analysis vendor	
Partek Genomic Solutions	www.partek.com
Agilent GeneSpring	www.chem.agilent.com/Scripts/PDS.asp?lPage=27881
dCHIP	biosun1.harvard.edu/complab/dchip/
RMA	www.bioconductor.org/
MAS 5 Affymetrix	www.affymetrix.com/index.affx
Bioconductor	www.bioconductor.org/
Bioinformatics vendor	
Ingenuity	www.ingenuity.com
Transfac	www.gene-regulation.com/pub/databases.html
DAVID/EASE	david.abcc.ncifcrf.gov/
Gene Expression Omnibus	www.ncbi.nlm.nih.gov/geo/
KEGG	www.genome.jp/kegg/
GenMAPP	www.genmapp.org/
Novartis SymAtlas	symatlas.gnf.org/SymAtlas/
Microarray World	www.microarrayworld.com/SoftwarePage.html (listing of software, platform, and other tools)