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Molecular Profiling of Antipsychotic Drug Function

Convergent Mechanisms in the Pathology and Treatment of Psychiatric Disorders

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Abstract

Despite great progress in antipsychotic drug research, the molecular mechanisms by which these drugs work have remained elusive. High-throughput gene profiling methods have advanced this field by allowing the simultaneous investigation of hundreds to thousands of genes. However, different methodologies, choice of brain region, and drugs studied have made comparisons across different studies difficult. Because of the complexity of gene expression changes caused by drugs, teasing out the most relevant expression differences is a challenging task. One approach is to focus on gene expression changes that converge on the same systems that were previously deemed important to the pathology of psychiatric disorders. From the microarray studies performed on human postmortem brain samples from schizophrenics, the systems most implicated to be dysfunctional are synaptic machinery, oligodendrocyte/myelin function, and mitochondrial/ubiquitin metabolism. Drugs may act directly or indirectly to compensate for underlying pathological deficits in schizophrenia or via other mechanisms that converge on these pathways. Side effects, consisting of motor and metabolic dysfunction (which occur with typical and atypical drugs, respectively), also may be mediated by gene expression changes that have been reported in these studies. This article surveys both the convergent antipsychotic mechanisms and the genes that may be responsible for other effects elicited by antipsychotic drugs.

Index Entries: Antipsychotic; neuroleptic; schizophrenia; gene expression; high throughput; microarray; myelin; synaptic.

Introduction

Neuropsychiatric disorders, including schizophrenia and bipolar disorder, are a heterogeneous group of devastating illnesses affecting 1 to 2% of the general population. The manifestations of schizophrenia include a diversity of both positive symptoms (such as delusions, hallucinations, disordered thinking, and bizarre behavior) and negative symptoms (including social withdrawal, lack of motivation, poverty of speech, and affective blunting) *(1)*. Cognitive

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Table 1
Effectiveness of Various Antipsychotic Drugs Against Clinical Symptoms in Schizophrenia and Their Propensity to Cause Side Effects

	Drugs used in gene profiling studies:		
Effects	Clozapine	Risperidone	Haloperidol
Positive symptoms	+++	+++	+++
Negative symptoms	++	+	–
Cognitive deficits	++	+	–
Treatment resistance	+++	+	–
Acute EPS	–	+/–	+++
Tardive dyskinesia	–	+	+++
Weight gain	+++	+/++	–
Hyperglycemia	++	+	–
Agranulocytosis	++	–	–
Neurotoxicity	++	–	+++

Data are taken from refs. *110* and *119*.

deficits are also observed in most patients. Many of these symptoms are also features of other psychiatric disorders. The efficacy of the presently available antipsychotic medications has been well-established in that they reduce symptomatology and prevent relapse in a large percentage of patients. The two main classes of drugs are the "typical" and "atypical" antipsychotics. Typical antipsychotic drugs, which include haloperidol, chlorpromazine, and fluphenazine, are primarily dopamine D_2 receptor antagonists. The "atypical" antipsychotics, such as clozapine, risperidone, quetiapine, and olanzapine, have a range of affinities for several different neurotransmitter receptors in addition to those for dopamine *(2–4)*. Atypical and typical antipsychotics are generally believed to be equivalent for the treatment of the positive symptoms of psychiatric disorders; however, studies have shown that clozapine exhibits superior clinical efficacy over typical antipsychotics *(5)*. Evidence also indicates that clozapine has beneficial effects on negative symptoms and cognitive deficits as well as for use with treatment-resistant patients (i.e., those who have not responded to conventional pharmacotherapies) *(4,6)*. The newer atypical antipsychotic medications are also usually preferred over older typical antipsychotic medications because of their more favorable side-effect profiles. Compared with the typical antipsychotics, the atypical drugs are associated with a lower incidence of extrapyramidal side effects (EPS) and tardive dyskinesia (TD) (Table 1). Unfortunately, despite this benefit, many atypical drugs (especially clozapine) are associated with metabolic side effects such as weight gain, hyperglycemia, and hypertriglyceridemia *(7–9)*. Clozapine is uniquely associated with the potentially deadly side effect of agranulocytosis (Table 1).

Mechanisms of Antipsychotic Drug Function

The major existing theories of psychiatric diseases were developed by exploring the mechanisms of action of these antipsychotic drugs. For example, the "dopamine hypothesis" of schizophrenia, formulated more than 30 yr ago, proposes that hyperactivity of dopaminergic neurotransmission causes symptoms of schizophrenia *(10, 11)*. Major support for this hypothesis comes from the excellent correlation observed between the clinical efficacy of typical antipsychotic drugs and their affinity for binding to the dopamine D_2 receptor *(12,13)*. Similarly, serotonergic pathways have also been

implicated in schizophrenia, largely because of the atypical drugs exhibit high affinity for the serotonin 2 class of receptors *(14,15)*. Mechanisms of drug action associated with these systems were reviewed previously *(16)*. There is a substantial body of evidence supporting neurotransmitter receptor abnormalities in psychiatric disorders, and this concept has successfully guided the development of drugs to treat these disorders. However, it has become clear that additional systems, components, and/or factors are required to explain the complex nature of these diseases and the mechanisms by which drugs act.

The advent of high-throughput expression profiling methods holds great promise for identifying gene expression changes that occur downstream from receptor blockade and for revealing novel molecular mechanisms that extend beyond hypothesis-driven research. Gene profiling methods allow for the simultaneous measurements of hundreds to thousands of messenger RNAs (mRNAs) in a given sample. To date, more than 10 papers have described experiments that have identified a plethora of gene expression changes in response to antipsychotic medications; the challenge is identifying which changes are most relevant. One approach is to consider at drug-induced changes in the context of psychiatric disorders by comparing findings from antipsychotic drug treatment to those identified in psychiatric disorders. These may be most relevant to symptom pathology. This article highlights important convergent mechanisms in disease and treatment and further cites evidence to implicate novel gene expression changes associated unwanted side effects.

Evidence for Gene Expression Alterations in Response to Drug Treatment

Considerable evidence suggests that the clinical effects of antipsychotic drugs are the result of changes in gene expression. First, imaging studies have shown that dopamine receptors are blocked more than 70% after only a few hours of antipsychotic administration, whereas relief of psychotic symptoms may take several weeks of drug administration *(17)*. The latency in the appearance of clinical benefit of drug administration likely results from the progression of a cascade of gene expression changes that gradually compensate for underlying neurochemical deficits in psychiatric disorders. Second, several studies have demonstrated activation of immediate early genes, such as fos and jun gene family members, after acute antipsychotic drug exposure *(18–22)*. Many immediate early genes are known to act as transcriptional regulators, thereby linking receptor-mediated effects to downstream changes in gene expression. Haloperidol and clozapine have been shown to induce different patterns of immediate early gene expression *(18–22)*, suggesting that different downstream targets are activated. Finally, prolonged exposure to typical antipsychotic drugs can induce TD, which may be persistent or often permanent *(23)*. Such permanent changes are certain to result from changes in gene expression.

Complexity of Gene Expression Changes in Psychiatric Disorders

Pathology-Driven Expression Changes

Schizophrenia is known to be a multifactorial disorder, with both genetic and environmental factors contributing to disease transmission. Several genes contribute to the inheritance of the disease *(24)*, but the environmental triggers remain unclear. Schizophrenia encompasses diverse and heterogeneous symptoms that may or may not be present in a given patient. Alterations in gene expression play a role in several aspects of disease presentation. Primary gene expression changes may result from genetic defects or environmental insults; however, these may not be present in all cases because of the heterogeneity of the disease. The expression of

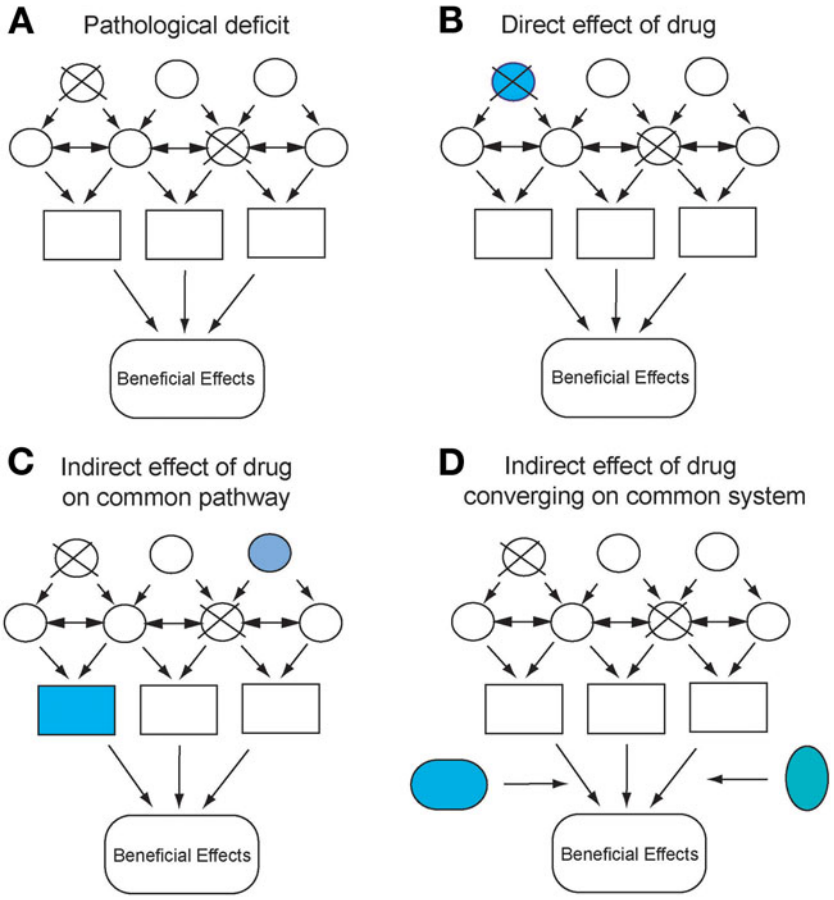

Fig. 1. Model of convergent mechanisms for drug actions. Circles and squares represent different genes that are part of a common pathway. Shaded components designate those targeted by drug treatment. "X" denotes a pathology-induced defect in a certain gene or in expression of that gene.

genes may also be altered as secondary response to a primary deficit. These secondary changes consist of compensatory or adaptational changes that attempt to restore normal functioning or, alternatively, may exacerbate pathology. Changes in gene expression may also be associated with additional environmental risk factors, generalized pathology, or specific symptoms that may be common to several psychiatric disorders. Because most, if not all, human postmortem brain samples are from subjects who had been exposed to antipsychotic drugs prior to death, the effect of treatment on gene expression profiles in psychiatric disorders may be a confounding variable.

Treatment-Related Changes

Antipsychotic drugs can elicit numerous complex changes in gene expression patterns in the brains of mentally ill patients. Gene expression changes may act directly or indirectly to compensate for underlying pathogenic deficits caused by a particular gene or dysfunction of a particular pathway or system (Fig. 1). Drugs may act directly on the same genes that have been implicated in the

Table 2
High-Throughput Gene Expression Profiling Studies Analyzing Effects of Antipsychotic Drugs

Species	Brain region	Drug; duration	Method	Reference
Rat	Cortex, striatum	Clozapine; 26 d Haloperidol; 26 d	Oligo. array	*45*
Rat	Substantia nigra VTA	Haloperidol; 21 d	Filter array	*47*
Rat	Cortex	Clozapine; up to 17 d Haloperidol; 17 d	cDNA array	*44*
Rat	Striatum	Haloperidol; 28 d	cDNA array	*46*
Rat	Striatum	Haloperidol; 28 d Clozapine; 28 d	Diff. Display	*120*
Rat	Cortex	Risperidone; 28 d	cDNA array	*81*
Rat	Cortex	Haloperidol; 28 d Risperidone; 28 d	Oligo. array	*82*
Rat	Striatum	Clozapine	cDNA array	*93*
Mouse	Cortex	Clozapine; 20 d	Filter array	*121*
Mouse	Cortex/striatum	Clozapine; 14 d Haloperidol; 14 d	TOGA	*67*
Mouse	Cortex	Haloperidol; 14 d Haloperidol + Biperiden	ATAC-PCR	*52*
Human	Glioma cells	Clozapine; 24 h Haloperidol; 24 h	Oligo array	*65*
Rat	PC12 cells	Chlorpromazine; 5 d	Filter array	*83*

VTA, ventral tegmental area; TOGA, Total Gene Expression Analysis; Oligo, oligonucleotide.

pathology of schizophrenia (Fig. 1B) or act on other genes in the same or similar pathways (Fig. 1C). Drugs may also target other components that converge on the same output system (Fig. 1D). These convergent mechanisms may represent the most important drug-induced changes targeting the underlying features of disease. Expression changes are likely to be responsible for the unwanted side effects that accompany treatment or for other detrimental effects. Antipsychotic drug-induced effects differ in different brain regions, depending on the type of drug used and the receptor profile for a given drug in the brain region studied. For example, typical antipsychotics, which are dopamine D2 receptor antagonists, might be expected to cause the greatest number of changes in the striatum, because of high dopamine D2 receptor density in this region.

High-Throughput Gene Profiling Studies on Rodents

In recent years, high-throughput analyses of the transcriptome have been applied to the study of the effects of antipsychotic drug effects in rodents (Table 2). The goals of these studies were to identify genes most important to mechanisms of drug treatment and to target pathways associated with the underlying symptoms that these drugs are used to treat. However, several factors make direct comparisons across these studies difficult: (1) the use of different methodologies; (2) different brain regions studied; and (3) various drug paradigms used.

First, two main types of high-throughput methodologies have been used to study drug effects: array-based methods, consisting of

complementary DNA (cDNA) and oligonucleotide microarrays and cDNA filter arrays and polymerase chain reaction (PCR)-based methods, such as differential display, Total Gene expression Analysis (TOGA®), and ATAC-PCR (Table 2). Explanations and considerations for the use of the different technologies and drawbacks in comparing datasets using similar methods have been reviewed elsewhere *(25,26)*, but essentially, the output of genes identified varies considerably among different methods. The nature of the different methodologies is one primary reason that the same pool of genes are not equally investigated; array-based methods allow for the measurement of expression differences among a predetermined set of genes, whereas PCR-based methods can identify any nucleotide sequence that binds to a particular primer sequence in a given pool of mRNAs. Although the coverage of the current technologies represents almost the entire expressed genome, most of the previous reports have screened only a fraction of the transcriptome.

Second, studies have investigated the effects of drugs on different brain regions, depending on the type of drug used or region of pathological interest (Table 2). The prefrontal cortex, which has been widely implicated in the pathophysiology of schizophrenia *(27)*, has been the most extensively studied area in human postmortem gene expression studies of schizophrenia. Therefore, rodent studies have examined cortex to correlate findings to human studies; these are the most useful for comparing expression differences. No high-throughput human postmortem studies have investigated striatum; however, this region has been widely studied in rodents because of the presence of dopamine receptors, which are a target for typical antipsychotic drugs. Postmortem studies have also investigated gene expression changes in hippocampus (another region associated with the pathophysiology of disease), but this region has not been studied in rodents. The different neurotransmitter receptor expression profiles characteristic of a certain region makes comparisons of studies performed on different brain regions difficult. It is also important to remember that the rodent cortex is comparatively different from primate. Interspecies differences in the cortex exist in the complexity of neuronal connectivity, the organization of dopaminergic innervation, and developmental processes *(28–30)*. The dynamics of dopamine function in the human striatum, which consists of a distinct caudate nucleus and putamen, also differs considerably from rodent striatum *(31,32)*. Therefore, the pharmacological response in cortical or striatal sites to antipsychotic drugs may be different in primate brain than in mouse brain, resulting in potentially different transcriptome profiles.

Finally, rodent studies have investigated the effects of both classes of drugs—typical and atypical antipsychotics—and have investigated different lengths of treatment. Comparisons of gene expression changes elicited by both types of drugs may reveal drug-common and -specific mechanisms. Both typical and atypical antipsychotic drugs are beneficial in treating the positive symptoms of the disease (Table 1). This effect has been attributed to blockade of dopamine receptors in the striatum, because increased limbic dopaminergic activity has been associated with the positive symptoms, and all antipsychotics have some affinity for dopamine receptors *(16)*. Therefore, the gene changes showing co-expression by both typical and atypical drugs may be preferentially associated with efficacy at treating positive symptoms. The most commonly studied drugs are haloperidol and clozapine, although two reports have examined expression changes in response to risperidone (Table 2). Gene expression changes unique to treatment with haloperidol likely represent those related to side effects, which are distinct from those observed after clozapine administration. Clozapine-specific effects on gene expression may be associated with several different effects, such as the superior therapeutic efficacy of clozapine, negative symptoms, treatment refractoriness, antisuicidal properties, or metabolic side effects.

Overall, high-throughput gene expression studies have reported a diversity of data, with many types of changes resulting from treatment with antipsychotic drugs. However, review of the literature reveals that gene expression alterations converge on the same systems as those identified to be important in the pathology of schizophrenia. Furthermore, examination of the region-specific gene expression patterns and gene ontology classification of genes reported to be altered by antipsychotics reveals genes that may be responsible for other aspects of drug exposure, such as side effects and neurotoxic effects.

Convergent Mechanisms in Pathology and Treatment

Several studies have used microarray analyses to assess gene expression profiles in human postmortem samples from subjects with schizophrenia. Data emerging from these studies have revealed many gene expression changes belonging to diverse systems that may be dysfunctional in schizophrenia. Concordant data sets have suggested that the most important areas associated with the pathology of schizophrenia are related to impairments in synaptic function, myelination, protein turnover, and energy metabolism. High-throughput studies have revealed that antipsychotic drug treatment regulates the expression of genes that converge on the same systems deemed most important to pathology of schizophrenia. Coregulation of genes by pathology and treatment may indicate that antipsychotic drugs are efficacious in schizophrenia because their effect on these systems improves the pathological changes observed in the disease.

Synaptic Machinery

The first system to emerge as dysfunctional in schizophrenia based on microarray studies on human postmortem brain was that involved in synaptic activity/communication. Mirnics and coworkers *(33)* identified significant decreases in the expression of multiple genes encoding presynaptic proteins in prefrontal cortex of schizophrenic subjects. Although dysfunction in this system appeared to be complex, with different subjects showing different alterations in presynaptic genes, the most consistently changed transcripts among 10 schizophrenic subjects were *N*-ethylmaleimide sensitive factor and synapsin II, whereas synaptogyrin 1, synaptojanin 1, and synaptotagmin V were decreased in some subjects *(33)*. Additional microarray studies that analyzed prefrontal cortex in subjects with schizophrenia substantiated these deficits *(34–36)*. The regulation of presynaptic-related transcripts is not limited to prefrontal cortex, because entorhinal cortical neurons from schizophrenic subjects also show decreases in synaptophysin and synaptotagmins I and IV *(37)*. Overall, these findings are consistent with studies using conventional methods that showed measured protein and mRNA expression levels of presynaptic proteins in prefrontal cortex and hippocampus of schizophrenic subjects *(38–43)*. The decreases identified in postmortem brain were not likely a result of typical antipsychotic drug exposure prior to death because chronic administration of haloperidol to rhesus monkeys showed no effect on transcripts related to synaptic function as a group in the frontal cortex *(33)*, and in the cortex of haloperidol-treated rats, no decreases in these proteins were detected *(44,45)*.

However, additional studies have shown that antipsychotic drugs exert a complexity of influences on the expression of genes encoding individual synaptic proteins in frontal cortex and striatum (Table 3; Fig. 2). Microarray analyses of cortical gene expression in rats have demonstrated that haloperidol and clozapine administration result in the induction of different synaptic transcripts *(44–47)*. Although many different variants or family members of a given synaptic gene group have been identified, several of the exact same transcripts have been shown to be regulated both in disease and by treatment (Fig. 2). One of the first cDNA array studies assessing the effects of

Table 3
Gene Expression Changes Elicited by Antipsychotic Drugs

Category	Typical antipsychotics	Atypical antipsychotics
Convergent effects		
Synaptic machinery	Synapsin II[a], Syn1a[b], Syn1b[b] Syn4[b], Syn8[b], VAMP1[b], VAMP2[b] SNAP-25[b], Syntag IV[b], SyntagXI[b], SyntagIII[b], Munc13[b], Syn5[b], Syn7[b], NSF[b], Rim1[b]	VAMP1[b], VAMP2[b], SNAP-25[b] Syn1a[c], Syn1b[b], Syn3[c], Syn4[b,c], Syn5[c], Syn8[b], Synaptophysin[c], SyntagII[c], SyntagV[c], SyntagVII[g], Munc18[c], SyntagXI[b,c], Rab1a[c], Rab3a[c] Rab8[c], Rab11a[c], Rab12[c], Rab13[c], Rab14[c], Rab26[c]
Oligodendrocyte/myelin dysfunction	HMGCR[d] HMGCS1[d]	ApoD[e], HMGCR[d], HMGCS1[d]
Protein degradation	UBA52[e], UBE2r2[e] UBE2E1[f], UBE2E2[f], UCHL5[f] UCHL1[f], UBE2B[f], UBE2D1[f] UBE2E3[f], UBP[f]	UBA52[e], UBE2r2[e] ubiquitin C[g] polyubiquitin[h]
Other effects		
Extrapyramidal Side effects	Striatin[e], OSBPL-9[e], PDE1B[e], Adenosine A2a receptor[a]	Hippocalcin[g]
Weight gain	FADS1[d], FASN[d]	ApoD[e], GIP[i], SCD1[d] FADS2[d], FASN[d], FADS1[d]
Neurotoxic effects		
Excitotoxic effects	GRM1a[j,b], GRIN1[j], GRIN2a[b], GRIN2c[j] Gria1[j], Gria3[j,g], Gria4[a,j], GRIK4[b,j] GRIK5[b,e,j], Calgranulin A[g], VSNL2[c]	GRIK5[e], Cacna1a[e], Cacng8[g] Chromogranin A[c], Prothymosin α[g]
Apoptosis	Prothymosin α[a,g], Calpain2[h], Cathespin[h]	

[a]From ref. *46*.

[b]From ref. *45*.

[c]From ref. *44*

[d]From ref. *65*.

[e]From ref. *67*.

[f]From ref. *52*.

[g]From ref. *82*.

[h]From ref. *81*.

[i]From ref. *93*.

[j]From ref. *47*.

Atypical drugs are clozapine or risperidone. Typical drug is haloperidol.

VAMP, vesicle-associated membrane protein; SNAP-25, synaptosomal-associated membrane protein of 25 kDa; Syn, syntaxin; Syntag, synaptotagmin; SVP synaptic vesicle protein 2b; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HMGCS1, 3-hydroxy-3-methylglutaryl-coenzyme A synthase-1; apoD, apolipoprotein D; UBA52, ubiquitin A-52 fusion protein; UBE, ubiquitin-conjugating enzyme; UCHL, ubiquitin C-terminal esterase L; UBP, ubiquitin binding protein; OSBPL-9, oxysterol binding protein like-9; PDE1B, phosphodiesterase 1B; GIP, glucose-dependent insulinotropic polypeptide; SCD1, stearoyl-CoaA desaturase; FADS1, fatty acid desaturase 1; FADS2, fatty acid desaturase 2; FASN, fatty acid synthase. GRIM1a, glutmate receptor metabotropic; GRIK, glutamate receptor, ionotropic, kainate; Gria, glutamate receptor, ionotropic, AMPA; GRIN, Glutamate receptor, ionotropic, *N*-methyl D-aspartate; VSNL2, visinin-like protein 2; Cacna1a, calcium channel, P/a type α1A; Cacng8, calcium channel, γ8.

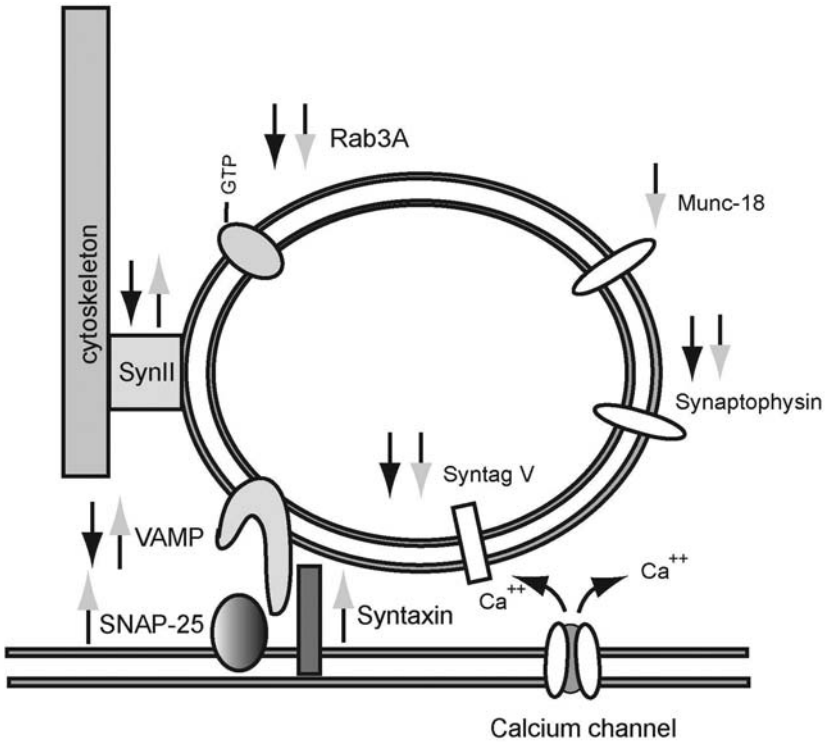

Fig. 2. Schematic depiction of a presynaptic neurotransmitter vesicle fusing with the plasma membrane upon influx of calcium (Ca^{++}) via calcium channels. Components important for vesicle mobilization and fusion are shown. Black arrows denote the direction of expression change for the given gene identified in post-mortem brains of subjects with schizophrenia. Gray-tipped arrows indicate the direction of expression change caused by treatment with antipsychotic drugs. SynII, synapsin; VAMP, vesicle-associated membrane protein; SNAP-25, synaptosomal-associated membrane protein of 25 kDa; SyntagV, synaptotagmin. (*See* Table 3 for references.)

chronic haloperidol on gene expression in rat striatum identified synapsin II as one of 14 significantly altered mRNAs *(46)*. Conversely to the decrease observed in schizophrenia, haloperidol caused an increase in synapsin II mRNA expression that resulted in the elevation of both synapsin IIa and IIb protein isoforms *(46)*. Follow-up studies demonstrated that haloperidol also elevated synapsin II levels in rat cortex *(48)*. In other microarray studies, no change in synapsin II was observed in response to haloperidol combined with clozapine *(45)* or clozapine alone *(44)*, indicating a specific effect for haloperidol. Functional linkage and association studies have indicated that synapsin II, located on chromosome 3p25, is an important susceptibility gene for schizophrenia *(49,50)*. Therefore, haloperidol may directly target a core deficit in schizophrenia. Evidence for a similar direct reciprocal effect of treatment on pathology exists for synaptobrevin (also known as vesicle-associated membrane protein [VAMP]), which is decreased in prefrontal cortex in schizophrenia *(39,51)* but increased by both haloperidol and clozapine in rat cortex *(45)*.

Other gene expression decreases observed in prefrontal cortex of schizophrenics that are not changed by haloperidol treatment are mimicked by treatment with clozapine. This is true for synaptophysin, synaptotagmin V and Rab3a, which have been shown to be downregulated in the cortex of schizophrenic subjects *(25,33,37)*. In a filter cDNA array study screening 1176

genes, the mRNAs encoding the same presynaptic proteins (synaptophysin, synaptotagmin V and Rab3a) were found by cluster analysis to be decreased *(44)*. It is possible that the decreases in the expression of these genes observed in schizophrenia represent a secondary response to pathology or a compensatory effect in response to a primary deficit in other synaptic genes, such as synapsin II or VAMP. The same direction of regulation of these three genes caused by clozapine and pathology may indicate that the brain's natural response is mimicked by clozapine. This may be one reason that clozapine is such an effective antipsychotic.

Also appearing on lists of regulated transcripts, or those screened but not changed, from several studies are other family members of synaptotagmins, Rabs, and syntaxins *(44,45,52)*. Synaptophysin, synaptotagmin V, and Rab3a are regulatory proteins that function in synaptic vesicle fusion and mobilization (ref. *53*; Fig. 2). Synaptophysin may play a role in the formation of a transient pore between the secretory vesicle and plasma membrane *(53)*. Synaptotagmins are believed to be calcium sensors, which respond to the influx of calcium from voltage-dependent calcium channels concentrated at active zones of presynaptic terminals *(53)*. The small G protein, Rab3A, targets synaptic vesicles to their release sites and is involved in vesicle recycling *(53)*. Other regulatory proteins, such as Munc18, also play important roles in vesicle fusion.

Alternatively, drugs may act indirectly on other components of a pathway to compensate for deficits in particular synaptic proteins. A recent oligonucleotide microarray study by MacDonald and colleagues *(45)* examined the expression of 8800 rat genes in response to haloperidol and clozapine and identified elevations in several mRNAs encoding presynaptic proteins. This study did not distinguish expression profiles elicited by each drug but focused on changes that were common to both drugs and, therefore, could account for the antipsychotic benefits attributed to the use of these drugs, minimizing attention to expression patterns responsible for side effects. Specific elevations in the expression of VAMPs 1 and 2, syntaxin 1b, and SNAP25 were found in the frontal cortex in response to haloperidol and clozapine, and these changes were verified by real-time PCR analysis *(45)*. Other syntaxin family members have been identified to be regulated by drug treatment; two different studies have reported elevations in syntaxin 8 *(44,45)* and an increase *(45)* and decrease in syntaxin 1A expression. Synapsin II anchors synaptic vesicles to the cytoskeleton (Fig. 2) and functions in the regulation of neurotransmitter release. VAMP 1 is the rate-limiting step in synaptic vesicle fusion, whereas VAMP 2, syntaxin 1, and SNAP25 comprise the minimal machinery needed to cause spontaneous membrane fusion *(54)*. Therefore, their elevation may play a pivotal role in facilitating synaptic activity in the context of decreased release of vesicles from the cytoskeleton resulting from low levels of synapsin II (Fig. 2). These findings suggest that this may be important to the therapeutic benefits of both haloperidol and clozapine. The accessory genes targeted specifically by clozapine may help explain why clozapine has been shown to exhibit superior clinical efficacy in treating positive symptoms in some patients.

Myelin-Related Gene Expression

Multiple lines of evidence now converge to implicate oligodendrocyte and myelin dysfunction in schizophrenia. Studies have shown reductions in white matter volume, decreased anisotropy of white matter tracts, regressive changes in oligodendrocyte structure and density, and decreases in the expression of myelin-related genes in the brains of subjects with psychiatric disorders (reviewed in ref. *55*). Hakak and colleagues *(56)* first reported decreases in myelin-related transcripts in postmortem prefrontal cortical samples from schizophrenic subjects, and since this report, several other genome-wide expression studies have substantiated these findings *(57–61)*. These deficits include mRNAs encoding myelin and lymphocyte protein, myelin-associated glycoprotein, proteolipid protein 1 (PLP1), myelin-associated oligoden-

drocyte basic protein, myelin oligodendrocyte glycoprotein, myelin basic protein, and 2′,3′-cyclic nucleotide 3′-phosphodiesterase *(57–59)*, which has recently been implicated as an important susceptibility gene in schizophrenia *(62)*. Dysregulation of myelin gene expression is not limited to prefrontal cortex; changes were also observed in middle temporal gyrus, superior temporal, and cingulate cortices and hippocampus *(60,61)*. No high-throughput studies on rodents have directly identified regulation of any of these myelin-related genes by antipsychotic drugs. However, in haloperidol-treated monkeys, a slight increase in PLP1 was observed *(57)*. Therefore, antipsychotic drugs are not likely to act at the level of myelin protein expression but may compensate for these deficits by indirectly targeting other points in the myelin pathway (*see* Fig. 1C). Myelin is essential for proper neuronal signal propagation and connectivity in the brain *(63)*. The production of myelin depends on synthesis of several myelin-specific proteins, such as those identified as downregulated in schizophrenia, as well as several lipid components. Evidence from gene profiling studies suggests that drugs, especially clozapine, may act to elevate lipid membrane components that may enhance myelin function under conditions of decreased myelin protein status.

Unesterified cholesterol is an indispensable component of myelin *(63)*, and it has been estimated that up to 70% of the brain cholesterol is associated with myelin *(64)*. A recent microarray study monitored the expression of 24,645 genes in human glioma cells after haloperidol and clozapine treatment *(65)*. At doses that did not cause cell death, exposure to haloperidol and clozapine for 3 to 24 h elicited elevations in the expression of genes involved in the synthesis of cholesterol, including the rate-limiting enzymes 3-hydroxy-3-methylglutaryl-coenzyme A reductase and 3-hydroxy-3-methylglutaryl-coenzyme A synthase-1 *(65)*. However, changes in enzyme levels resulted in an elevation of cellular content of cholesterol only after clozapine treatment, indicating a differential effect of the two drugs *(65)*. Cholesterol enrichment of myelin would lead to increased myelin function and signal propagation down the axon *(63)*. Furthermore, cholesterol also plays a vital role in the development, function, and stability of synapses *(66)*, thus altered levels of cholesterol may be associated with changes in presynaptic activity.

Our previous studies also provide evidence for a clozapine-specific effect that may enhance myelin function. Using the PCR-based methodology, TOGA, we screened approx 17,000 transcripts in mouse cortex and striatum for regulation by clozapine and haloperidol *(67)*. Among 51 mRNAs identified, apolipoprotein D (apoD) mRNA stood out as specifically regulated by clozapine but not by haloperidol *(67,68)*. Studies by others also showed that in addition to clozapine, apoD was elevated in response to the atypical antipsychotics olanzapine and risperidone *(69–71)*. ApoD, a 29-kDa glycoprotein, is most abundantly expressed in oligodendrocytes, the myelin-producing cells in the brain. ApoD has been shown to bind with high specificity and affinity to arachidonic acid *(72,73)*. In addition to the specific myelin proteins mentioned previously, myelin is composed of phospholipids and sphingolipids, which are ester or amide derivatives of glycerol or sphingosine that contain fatty acids. Arachidonic acid is a major fatty acid contained in membrane phospholipids, including myelin membranes *(74)*. In vitro studies have shown that apoD enhances incorporation of arachidonic acid into membranes and prevents the release of arachidonic acid into the cytosol *(75)*. The role of apoD and arachidonic acid in psychiatric disorders has been discussed in greater detail previously, where we have suggested that elevated levels of apoD caused by treatment with atypical drugs would result in a stabilization of membrane fatty acids *(76)*. This effect in oligodendrocyte membranes would be essential to proper myelin function. This system may be especially relevant to therapeutic benefits of clozapine because white matter deficits have been associated with negative symptoms *(77,78)* and clozapine is effective against negative symptoms (Table 1).

Ubiquitin-Protease System/Metabolism Dysfunction

Based on the decrease in expression of genes related to the ubiquitin protein degradation system, a dysfunction in protein turnover has been implicated in schizophrenia. Ubiquitin is a small protein composed of 76 amino acids that is highly conserved among eukaryotes. Ubiquitin functions to regulate protein turnover by tagging proteins for degradation by the 26S proteasome. Microarray studies on human postmortem tissue from schizophrenics have demonstrated deficits in this system. Decreases in the expression of ubiquitin-specific proteases 2, 9, and 14, ubiquitin C-terminal esterase L1 (UCHL1), ubiquitin-conjugating enzyme (UBE)2N, UBE2H, and ubiquitin ligase 3b (UBE3b) in the prefrontal cortex of schizophrenic subjects *(34,51,79)*. Decreases in expression have also been observed in dentate granule neurons of the hippocampus in UCHL1, UBE2D1, UBE2D3, ubiquitin B, and several other genes encoding proteasome subunits *(80)*. There is indication that antipsychotic drugs also act on this system, although they act in a complex manner. Conversely to the decreases observed in subjects with schizophrenia, antipsychotic drugs have been shown to elevate the expression of certain ubiquitin-related genes in mouse brain. Atypical drugs elicit elevations in the levels in the mRNAs of ubiquitin A-52 fusion protein (UBA52) *(67)* and polyubiquitin *(81)* in the cortex, which may help combat pathological deficits in this system. Haloperidol and clozapine increased UBA52 and UBE3b in the striatum, and the latter is elevated almost sevenfold by haloperidol treatment.

Other microarray studies have shown decreases in the expression of ubiquitin transcripts by many types of drugs. A decrease in ubiquitin C was observed by risperidone in rat cortex *(82)*. In another report examining the effects of co-administration with haloperidol and biperiden (an antimuscarinic drug used to help suppress extrapyramidal side effects), decreases in the average levels of several ubiquitin-related transcripts were identified: UBE2B, UBE2D1, UBE2E1, UBE2E2, UBE1, UCHL1, and UCHL5. However, no significant decreases were observed with either drug alone *(52)*. This system is important for the complex regulation of protein degradation, but depending on the substrates for the different enzymes, increases and decreases in expression of particular components do not clearly translate into beneficial or detrimental effects.

Converging sets of metabolic transcriptional abnormalities have also been identified from drug gene profiling studies on rodents *(45,67,82,83)* and microarray studies on human postmortem brain from schizophrenics *(34,79,80)*, especially deficits in mitochondrial function. Mitochondria dysfunction in schizophrenia and its treatment has been reviewed elsewhere *(84)* and will not be discussed in detail here. However, a few key findings that have been identified in recent studies include elevations in cytochrome C reductase expression in response to clozapine and haloperidol in the striatum *(67)* and an increase in cytochrome C oxidase in PC12 cells treated with chlorpromazine *(83)*. Overall, these studies provide evidence that drugs may help normalize brain metabolism through actions on the ubiquitin and mitochondrial systems.

Side Effects of Atypical and Typical Drugs

Extrapyramidal Side Effects

The striatum is the largest and major receptive component of the basal ganglia, a group of subcortical nuclei that include the substantia nigra, globus pallidus, and the subthalamic nucleus. The striatum is critically involved in the generation of directed motor behaviors and the initiation of movement *(85)*. Long-term treatment with typical antipsychotic agents is associated with the development of TD and other EPS that result from prolonged blockage of dopamine receptors in the striatum *(22)*. These motor side effects, which affect more than 60% of the people who take conventional

antipsychotic medications, can cause various symptoms, such as involuntary movements, Parkinsonism, tremors and rigidity, akathisia, and dystonia.

We have previously hypothesized that mRNAs with restricted expression in the striatum are likely to encode proteins that are preferentially associated with particular physiological or behavioral processes in the striatum *(86)*. In other words, genes with predominant expression in the striatum are more likely to be associated with motor/movement functions than genes expressed ubiquitously or in other regions. In our high-throughput screen of genes regulated by clozapine and haloperidol in mouse striatum *(67)*, three genes were identified that exhibit predominant expression in the striatum: phosphodiesterase 1B (PDE1B) *(87)*, striatin *(88)*, and oxysterol binding like protein-9 (OSBPL-9) *(67)*. Although these genes also show moderate expression in the cortex, expression changes in response to drug treatment were only detected in the striatum. Striatin was found to be elevated specifically by haloperidol treatment. PDE1B and OSBPL-9 were elevated by both types of drugs; however, greater changes were observed by haloperidol *(67)*. Using cDNA expression arrays, another mRNA that is preferentially expressed in the striatum, the adenosine 2A receptor *(89)*, was found to be significantly decreased by haloperidol administration in rat striatum *(46)*. Hippocalcin, another mRNA with striatal-enriched expression *(90)*, was found at lower levels in the cortex after risperidone administration *(82)*. Despite being classified as an atypical antipsychotic drug, risperidone does show propensity toward EPS because of its high affinity for dopamine D2 receptors (Table 1). Increases or decreases in the expression of these genes in the striatum may be particularly important to the development of unwanted motor side effects and could be targeted for therapeutics to combat such side effects.

Weight Gain and Other Lipid Abnormalities

Long-term administration of many atypical drugs, but not the typical antipsychotics, can result in excessive weight gain as well as increased levels of triglycerides, leptin, and insulin *(8,91,92)*. Such consequences can affect up to 50 to 85% of patients taking atypical antipsychotics, especially clozapine *(8,91,92)*. From the published gene profiling studies showing changes in gene expression elicited by clozapine, there is an indication that certain genes may be associated with these effects.

Using cDNA arrays, researchers found that chronic treatment with clozapine resulted in upregulation of the glucose-dependent insulinotropic polypeptide (GIP) transcript by more than 200% in the rat striatum *(93)*. Interestingly, GIP appeared in another gene profiling screen as decreased by haloperidol *(46)*, which does not cause weight gain. Their further analyses found that, in addition to brain, GIP mRNA levels in the small intestine and the plasma were significantly elevated in subjects treated with clozapine *(93)*. GIP is an insulinotropic agent with stimulatory effects on insulin synthesis and release from the pancreas. Its actions in the periphery, which mimic that observed in the central nervous system, may lead to hyperinsulinemia and diabetes.

In addition to the potential effects on myelin described earlier, apoD may also be associated with clozapine-induced weight gain and lipid abnormalities *(68)*. Apolipoproteins play an important role in the maintenance of lipid homeostasis throughout the body. In the plasma, apoD is associated with high-density lipoproteins (HDLs) *(94)*, which also contain phospholipids, cholesterol, and fatty acids. Obesity is frequently associated with high plasma triglyceride and reduced plasma HDL cholesterol levels *(95,96)*. As a component of HDLs, apoD levels in the periphery may be altered in obesity. However, a recent report suggested potential central effects of apoD in response to increased body fat. Mice fed a high-fat diet exhibited increases in apoD expression in the hypothalamus *(97)*, a region of the brain essential in the coordination and regulation of food intake, body fat regulation, energy expenditure, and overall energy homeostasis *(98,99)*.

ApoD has also been shown to bind the leptin receptor in the hypothalamus *(97)*. Leptin mediates many actions, such as inhibition of food intake, increased energy expenditure, promotion of fat oxidation, and homeostasis of lipids via interactions with specific leptin receptors *(100–102)*. A further link between apoD and body fat comes from studies demonstrating linkage of apoD polymorphisms to obesity and type II diabetes *(103,104)*.

The microarray study by Ferno and coworkers *(65)* showed that human glioma cells exposed to antipsychotic drugs resulted in elevations in transcripts involved in lipogenesis, and these could be very important to mechanisms of weight gain. Clozapine-specific increases were identified for the gene encoding fatty acid desaturase and stearoyl-CoA desaturase-1 (SCD-1), the enzyme that converts saturated long-chain fatty acids to monounsaturated long-chain fatty acids *(105)*. Monounsaturated long-chain fatty acids are more readily incorporated into triglyceride than saturated fatty acids; therefore, elevated enzyme levels could lead to hypertriglyceridemia. Recent studies have also demonstrated that elevated levels of SCD-1 contribute to abnormal lipid metabolism and progression of obesity *(106)*. Another molecule, fatty acid synthase, which was elevated 50% greater by clozapine than haloperidol, plays a central role in *de novo* lipogenesis and triacylglycerol storage into adipose tissue *(107)*. Additionally, inhibition of fatty acid synthase results in a decrease in food intake and body weight in rodents *(108)*; therefore, an increase in expression may have the opposite effect.

Changes in expression of the genes described earlier may account for not only weight gain but the elevated levels of insulin, diabetes type II, and triglycerides observed after clozapine treatment. Because weight gain and lipid abnormalities can be dangerous to patients' health and are main reasons for patient noncompliance, identification of novel targets to address these issues is an important research focus.

Neurotoxic Effects of Antipsychotics

From examining neuropathological changes in brains of patients exposed to antipsychotic drugs, in 1977, drugs were first suggested to have neurotoxic effects *(109)*. Since then, several studies have reported a range of toxic effects in response to drug exposure, including cell death, DNA fragmentation, nuclear membrane disintegration, and apoptosis (reviewed in ref. *110*). Toxic effects caused by haloperidol have long been reported, although the mechanism is uncertain. Haloperidol may cause cell death via excitotoxic, apoptotic, or oxidative stress-related pathways. It was initially believed that excitotoxic effects caused by typical antipsychotics were important to the generation of extrapyramidal side effects *(111)*; however, the finding that clozapine also shows neurotoxic properties *(110,111)* suggests that these effects may contribute to an exacerbation of symptomatology. Interestingly, neurotoxic properties are not observed for other atypical drugs, such as risperidone *(111)*.

Some of the toxic effects of drugs may represent an excessive attempt to restore a hypoglutamatergic state in schizophrenia. The "glutamate hypothesis" of schizophrenia has come from the observations that glutamate receptor antagonists exacerbate psychotic symptoms in schizophrenics *(112)* and produce cognitive deficits and psychotic symptoms in healthy volunteers, similarly to schizophrenia *(113)*. Accordingly, several studies have reported abnormal glutamate receptor binding in postmortem brain samples from subjects with schizophrenia (reviewed in ref. *114*), and microarray analysis has specifically demonstrated decreases in transcripts related to glutamatergic neurotransmissions in prefrontal cortex of schizophrenics *(33)*. Antipsychotic drug transcriptome profiling studies have demonstrated that haloperidol increases mRNAs encoding glutamate receptor subunits in rat substantia nigra and ventral tegmental area *(47)*. Importantly, three separate reports showed increases in the ionotropic glutamate receptor, kainate 5 in expression of the response to

haloperidol but not clozapine (refs. *45, 47* and *67*; Table 3). These effects may help compensate for decreased glutamatergic activity; however, too much "compensation" could be detrimental. Whereas the physiological stimulation of glutamate receptors is beneficial for neurons, excessive stimulation of glutamate receptors can cause excitotoxicity, which is triggered by elevated levels of intracellular calcium *(114,115)*. This can also lead to calcium-dependent apoptotic death or programmed cell death, which is associated with activation of intracellular proteases *(115,116)*. Furthermore, drug gene profiling studies have reported alterations in levels of several mRNAs related to calcium signaling and homeostasis, which may also be associated with excitotoxic and apoptotic mechanisms. These include changes in calcium channel subunits *(67,81,82)* and calcium-binding proteins (refs. *44,67,81,82*; Table 3). Alternatively, some of these changes may be beneficial to patients as they may relate to improved synaptic function (Fig. 2). For example, visinin-like protein 2 whose expression is elevated by the haloperidol is believed to act as calcium sensor, which may play a role in neurotransmitter release *(117)*.

Apoptotic cell death may also result from altered levels of anti-apoptotic proteins. Two separate reports have demonstrated that haloperidol decreases the expression of prothymosin-α in rat striatum and cortex *(46,82)*. This proteins acts as an inhibitor of the apoptosome; therefore, decreased levels caused by drug treatment could disrupt normal regulatory processes leading to apoptosis *(118)*. Overall, expression changes in genes related to the glutamate and calcium systems likely account for the potentially toxic effects of antipsychotic drugs. These systems are particularly affected by haloperidol, which elicits a greater range of neurotoxic effects.

Impact for Molecular Psychiatry

The past few years have brought much progress in our understanding of mechanisms of antipsychotic drug action. Evidence suggests that in addition to targeting neurotransmitter systems, drug-induced expression changes are converging on the same systems deemed most important to the pathology of psychiatric disorders, such as dysfunctions in synaptic machinery, myelin function, and protein metabolism. These systems may represent the most appropriate pathways to target for future therapeutic because actions on neurotransmitter systems, such as enhancing glutamatergic activity or blockage of dopaminergic signaling, can result in detrimental side effects. Furthermore, our understanding of genes that may be associated with unwanted side effects of drug treatment should lead to approaches that will prevent or reduce the occurrences of these in the future.

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