Multifunctional Pharmacotherapy: What Can We Learn from Study of Selective Serotonin Reuptake Inhibitor Augmentation of Antipsychotics in Negative-Symptom Schizophrenia?

Henry Silver,* Yael Chertkow,* Orly Weinreb,[†] Lena Danovich,* and Moussa Youdim[†]

**Molecular Neuropsychiatry Unit, Brain Behavior Laboratory, Shaar Menashe Mental Health Center, and Technion–Faculty of Medicine, Haifa 32000, Israel, [†]Eve Topf Center of Excellence for Neurodegenerative Diseases Research, Department of Pharmacology, Technion–Faculty of Medicine, Haifa 32000, Israel*

Summary: Many patients suffering from major psychiatric disorders do not respond adequately to monotherapy and require additional drugs. To date, there are no objective guidelines for deciding which combination may be effective, and the choice is based on previous clinical experience and on trial and error. Even when combination drugs are effective, the biochemical mechanisms responsible for the value-added effect are unknown. Understanding the mechanism of such synergism may provide a rational basis for choosing drug combinations and for developing more effective drugs. In schizophrenia, negative symptoms respond poorly to antipsychotics, but may improve when these are augmented with selective serotonin reuptake inhibitors (SSRI). This augmenting effect cannot be explained by summing the pharmacological effects of the individual drugs. We proposed that the study of SSRI augmentation can serve as a window to understanding the biochemical

mechanisms of clinically effective drug synergism. In a series of studies we identified unique biochemical effects of the combination, different from each individual drug, and proposed that some of these are involved in mediating the clinical effect. Here we review some of the findings and propose that the mechanism of action involves regionally selective modulation of the GABA system. The evidence indicates that the SSRI antidepressant–antipsychotic combination may be a useful paradigm for studying therapeutically effective synergistic drug interactions in schizophrenia. Although as yet limited in scope, the findings of definable molecular targets for synergistic SSRI–antipsychotic interaction provide new directions to inform future research and provide novel bio-molecular targets for drug development. **Key Words:** Schizophrenia, negative symptoms, selective serotonin reuptake inhibitors, antipsychotic drugs, GABA.

AUGMENTATION STRATEGIES IN PSYCHIATRIC DISORDERS

Psychiatric disorders such as schizophrenia, affective disorder, and obsessive compulsive disorder, are characterized by a wide range of behavioral, emotional, and cognitive abnormalities that often do not resolve completely with treatment.^{1–3} Development of new drugs over the last few decades has not produced dramatic improvement in response of schizophrenia, as highlighted recently by findings of large comparative studies.^{4,5} Negative and cognitive symptoms of the illness have proved particularly resistant to treatment. In clinical practice, this often results in the use of more than one

drug and, over the years, many combinations of psychotropics have been tried in an attempt to improve response. There is evidence that some combinations do induce a favorable clinical response not achieved by each of the drugs given alone, in some patients,^{2,3,6,7} but the choice of drug combinations and indications for their use are based on a trial-and-error paradigm guided by clinical experience and response.

Thus, there are empirical as well as theoretical reasons to think that single-action “magic bullets” may not be sufficient to treat complex neuropsychiatric diseases such as schizophrenia, and that multifunctional drugs or drug combinations that can simultaneously modify a number of CNS targets may be more effective. Better understanding of the biological principles by which combined treatments produce value-added effects can provide a rational basis for selection of appropriate drugs, as well as providing insights into the pathological mecha-

Address correspondence and reprint requests to: Professor H. Silver, Brain Behavior Laboratory, Shaar Menashe MHC, Mobile post Hefer 38814, Israel. E-mail: mdsilver@tx.technion.ac.il.

nisms of the disorders. In this review, we examine some recent studies of SSRI augmentation of antipsychotics that have been demonstrated in controlled clinical trials to ameliorate negative symptoms in schizophrenia, and we examine their potential to serve as windows to understanding the molecular mechanisms underlying synergistic drug interactions in treatment of schizophrenia and other psychiatric disorders.

SELECTIVE SEROTONIN REUPTAKE INHIBITOR-ANTIPSYCHOTIC THERAPY FOR NEGATIVE SYMPTOMS IN SCHIZOPHRENIA

Schizophrenia is a chronic, disabling brain disorder that affects some 1% of the population worldwide and places a severe burden on the sufferer, the family, and society. The symptoms can be divided into two groups: positive symptoms (e.g., hallucinations and delusions) and negative symptoms (e.g., apathy, anhedonia, flat affect, avolition, and social withdrawal). Negative symptoms in particular are a challenge to available treatments. Antidopaminergic drugs, the mainstay of antipsychotic treatment, are usually effective against positive symptoms but not against negative symptoms; the success of newer generation, atypical drugs is also limited.^{5,8}

A number of studies have demonstrated that adding SSRI antidepressants to antipsychotics can improve negative symptoms resistant to antipsychotic alone. Improvement was noted in controlled studies in patients treated with SSRI added to typical antipsychotics^{9–12} and in open studies and case reports on SSRI augmentation of atypical drugs (clozapine and olanzapine).^{13–16,18} The response is in negative symptoms primary to the illness,¹⁹ requires a serotonergic mechanism,¹² and can be detected within 2 weeks of starting augmentation.²⁰

The mechanism of SSRI augmentation is unknown. Several SSRI antidepressants inhibit cytochrome P-450 enzymes, mainly CYP1A2 and CYP2D6 isozymes, which are metabolizers of antipsychotic drugs.^{21–23} Adding an SSRI antidepressant drug often results in an increase in plasma levels of the antipsychotic,^{24,25} which could underlie the superior efficiency of the augmentation treatments. However, improvement in negative symptoms of schizophrenic patients treated with SSRI and haloperidol did not correlate with the increase of antipsychotic levels in plasma.^{26,27} Increasing antipsychotic drug levels does not increase the overall efficacy or the range of responsive symptoms, and may have adverse effects.^{28–30} Antidepressant doses used in antidepressant–antipsychotic combinations are usually lower than those used in monotherapy.⁷ Augmentation with SSRIs^{24,25,31} or other drugs^{32–34} that have no pharmacokinetic interactions with antipsychotics can also be effective in schizophrenia patients. Thus, pharmacokinetic interactions cannot explain the clinical efficacy of SSRI

augmentation strategies for negative symptoms in schizophrenia.

The contribution of SSRI drugs as augmentation agents for treatment of negative symptoms appears to be distinct from nonspecific antidepressant action. First, in several controlled clinical studies, patients with prominent depressive symptoms were excluded and depression scale scores of participants were low and did not change following augmentation treatment.⁷ Second, tricyclic antidepressants, which are equally effective antidepressants, do not improve negative symptoms in schizophrenic patients, although they can be useful when depression is associated with the disease.³⁵ Notably, SSRIs (which acts through serotonergic receptors), but not maprotiline (which acts via noradrenergic receptors), are effective augmentation agents for negative symptoms,¹² which suggests that the mechanism requires modification of the serotonergic system.³⁵ Paradoxically, clozapine and fluvoxamine–haloperidol combination produce a similar clinical effect, despite their different pharmacology: clozapine is a 5-HT antagonist and fluvoxamine elevates 5-HT concentration. Furthermore, adding SSRI to clozapine may improve effectiveness.^{16,17}

Thus, the mechanisms mediating the clinical effects are likely to be located downstream from the initial pharmacological effects of the drugs at the receptor–transporter levels.

THE SSRI-ANTIPSYCHOTIC COMBINATION AS A PARADIGM FOR INVESTIGATING DRUG-DRUG SYNERGISM

Several characteristics of the SSRI antidepressant–antipsychotic combination make it a useful paradigm for studying therapeutically effective synergistic drug interactions in schizophrenia. First, the clinical effect is specific for a particular subset of clinical symptoms (negative symptoms), one that constitutes a core dimension of the illness,^{1,36} with a response detectable within 2 weeks. Second, it is dependent on serotonergic action of the SSRI but not due to a general antidepressant effect. Third, the molecular mechanisms cannot be explained by the known actions of the individual drugs. Indeed, the fact that both clozapine, a serotonergic antagonist, and SSRI, a serotonin agonist, are effective is paradoxical, and although the pharmacological properties predict that combining clozapine with SSRI will lead to antagonism, clinical studies show that effectiveness may be improved.^{9,37}

To account for these characteristics, we hypothesized that chronic treatment with the SSRI–antipsychotic combination produces unique biochemical changes in the brain, different from those of the individual drugs, and that these mediate the enhanced clinical effectiveness.³⁷

We argued that molecular changes unique to the drug combination and different from the action of individual drugs encompass those changes that are relevant to clinical effectiveness of the combined treatment and to the psychopathology of negative symptoms. We further argued that, by comparing the changes unique to the combination to those caused by clozapine, a chemically distinct drug also effective against negative symptoms, we could further narrow the focus on the molecular changes relevant to the clinical efficacy against negative symptoms. We argued that this clinically effective treatment could serve as the guiding framework to help identify clinically relevant molecular changes in the laboratory and provide a novel paradigm to study molecular mechanisms relevant to schizophrenia.

MOLECULAR MECHANISMS OF SSRI-ANTIPSYCHOTIC SYNERGISM

From this perspective, we investigated the effects of SSRI-antipsychotic combination on molecular systems in the rat brain. We chose fluvoxamine, the SSRI most frequently studied in clinical trials, and haloperidol, the classic antidopaminergic antipsychotic, as the study drugs. The standard experiment compared five groups: fluvoxamine and haloperidol, haloperidol alone, fluvoxamine alone, vehicle, and clozapine. Because the clinical effect is not immediate, we concentrated on chronic (30 days) and subchronic (14 days) administration and examined several brain regions including frontal cortex, striatum, and hippocampus.

Monoamine systems

Chronic administration of haloperidol plus fluvoxamine produced unique changes in dopaminergic and serotonergic metabolism in the rat brain, not shared by the individual drugs.³⁸ The effects were regionally selective and included areas known to be relevant to schizophrenia pathology. For example, haloperidol combined with fluvoxamine reduced dopamine turnover in the cerebellum, an area implicated in the pathophysiology of schizophrenia.³⁹ Notably, clozapine showed a similar effect.

The combined haloperidol and fluvoxamine treatment increased dopamine metabolites and tyrosine hydroxylase protein level in the striatum when measured 1.5 hours after the last dose of chronic (30 days) treatment.³⁸ The effect of the combined treatment on dopamine metabolism was significantly greater than that of haloperidol alone and only in the combined group was this associated with changes in tyrosine hydroxylase protein levels. The authors interpreted this as indicating that adding fluvoxamine modulates the effect of haloperidol on dopamine metabolism. Because, like other SSRIs, fluvoxamine does not block dopaminergic receptors,⁴⁰ inhibit MAO activity,⁴¹ or directly affect dopamine turn-

over,^{38,42,43} it is likely that, when combined with haloperidol, fluvoxamine modifies dopamine pathways indirectly, perhaps via the serotonergic system.⁴⁴ These effects appear to depend on chronic administration, given findings from acute studies of increased dopamine release in rat prefrontal cortex after 5-HT uptake inhibitors combined with haloperidol, an effect not produced by the individual drugs,^{45–48} but no change in dopamine metabolism^{49,50} in the striatum.

GABA system

In a chronic (30 days) administration study, Chertkow et al.⁵¹ used cDNA arrays to screen a broad range of genes in the frontal cortex. Prominent alterations were then analyzed in real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and their related proteins examined by the Western-blotting technique. The gene expression profile after fluvoxamine plus haloperidol administration was different from that of fluvoxamine or haloperidol given alone. Notably, clozapine showed some homology with the combined treatment. The protein expression changes unique to the combined treatment included glutamic acid decarboxylase (GAD₆₇) and protein kinase C β (PKC β). The latter showed a similar trend following clozapine treatment. These findings support the hypothesis that SSRI-antipsychotic combination produces unique changes, different from those due to the individual drugs, and suggests that the mechanism of action may involve modification of the gamma aminobutyric acid (GABA) system.⁵¹ Further studies in which the medications were administered for 14 days also supported this hypothesis. The combination of fluvoxamine and haloperidol resulted in unique changes in several elements of the GABA system in the rat frontal cortex, including GABA_AR β 3, GAD₆₇, PKC β 2, and ERK, which differed from the effect of each drug given alone (Danovich et al.⁵² and unpublished data).

The unique molecular changes observed following combined treatment could not be explained by pharmacokinetic interactions, given that the concentrations of haloperidol and fluvoxamine in the brain and serum of the treated animals were similar whether the drugs were administered alone or in combination.³⁸

The observed molecular differences between treatment groups appeared in parallel with changes in overt behavior. Behavioral assessment performed a day before the animals were euthanized for post mortem analysis showed that animals in the haloperidol plus fluvoxamine group differed in motor activity and social behavior (sniffing) from those administered haloperidol or fluvoxamine alone.³⁸ The authors interpreted this as support for the view that changes observed in the brain may have functional significance.

GABA HYPOTHESIS OF SSRI-ANTIPSYCHOTIC AUGMENTATION

Based on these and other findings, we hypothesize that the molecular signaling pathways that regulate GABA receptor function (e.g., protein kinases C and A [PKC, PKA] and mitogen activated protein kinase [MAPK]) modify reciprocal interactions among dopamine, serotonin, and GABA neurotransmission and that their modulation mediates the clinical effect of SSRI augmentation on negative symptoms. The effect is localized to the prefrontal cortex (or its circuits) and is relevant to negative symptoms and (perhaps) cognitive impairments. It is further proposed that different mechanisms are responsible for the therapeutic effect on positive symptoms.

CLINICAL RELEVANCE

The relevance of laboratory findings and consequent hypotheses to the clinical situation requires independent validation in patients. One way to examine this is to study expression of gene products and proteins in blood cells of patients undergoing augmentation treatment. Peripheral mononuclear cells may reflect molecular processes in the CNS of schizophrenic patients and have been used to study various substances including cytokines (e.g., interleukins IL-6, IL-10, and IL-2), receptors for major neurotransmitters (e.g., dopamine, serotonin, and GABA_A), and G-protein subunits that regulate intracellular signal transduction.^{53–61}

Chertkow et al.⁶² used microarrays to examine the gene expression profile of peripheral mononuclear cells in schizophrenia patients on steady antipsychotic treatment before and 3 and 6 weeks after addition of fluvoxamine and found changes in transcripts related to G-protein coupled receptors. Using customized cDNA array and real-time RT-PCR, they also found significant downregulation of chemokine receptors IL8RA and CCR1, and of RGS7 mRNA. Notably, these changes paralleled improvement in negative symptoms. Several of the peripheral mononuclear cell genes that changed in the course of augmentation treatment have been reported to be abnormally expressed in post mortem brains of schizophrenia patients. These include genes for the 5-HT_{2A} receptor,⁶³ IL-1 receptor antagonist,⁶⁴ RGS7,^{65,66} and the neural specific protein neurogranin.⁶⁷ Although one must keep in mind the limitation of using peripheral changes as indicators of brain processes, these findings nonetheless support the view that these laboratory findings may have clinical validity.

The hypothesis that SSRI-haloperidol augmentation acts by modulating GABA system is consistent with accumulating evidence of GABA system abnormalities in schizophrenia. Post mortem studies show loss of specific GABAergic inhibitory neurons,⁶⁸ reduction in the

GABA synthesizing enzyme (GAD₆₇)^{69–72}, and compensatory upregulation of the GABA_A receptor^{73,74} in brains of schizophrenia patients. In addition, hypofunction of the ionotropic glutamate NMDA receptor⁷⁵ and its modification⁷⁶ may play an important role in the pathophysiology and treatment of negative symptoms, respectively, and lead to inhibition of GABAergic interneurons.⁷⁷

GABA plays a key role in the function of the prefrontal cortex, an area impaired in schizophrenia⁷⁸ and linked to negative symptoms and cognitive impairment,^{79,80} including working memory, a core cognitive deficit in the illness.⁸¹ Antipsychotic drugs can modify GABA and glutamate system elements, including GABA_A receptor, extracellular GABA levels and expression, glutamate transporters,⁸² and neuregulin-1 (which regulates the expression of NMDA and GABA_A receptors),⁸³ as well as ionotropic and metabotropic glutamate receptors.^{84,85} Interestingly, atypical antipsychotics differ from the typical ones in their effect on the GABA system, including hippocampal and cortical GABA_A receptor density,⁸⁶ GABA transporter expression,⁸⁷ and GABA transporter levels.⁸⁸ Mechanisms that may mediate drug-induced modification of the GABA system include inhibition of dopamine innervations on GABA and glutamate neurons, activation of PKC anchored to Rack1 (which then phosphorylates GABA_A receptor),⁸⁹ and increase in brain levels of allopregnanolone, a potent positive allosteric modulator of GABA at the GABA_A receptor.⁹⁰ Several of these elements showed unique changes following combined SSRI-antipsychotic treatment.

There is clinical evidence linking GABA/glutamate active agents with negative and cognitive symptoms in schizophrenia. Silver et al.⁹¹ found significant correlations between blood levels of dehydroepiandrosterone in its sulfated form (DHEAS) levels and cognitive function in schizophrenia patients. Augmentation with DHEA⁹² or agonists at glycine sites^{93,94} have been reported to improve negative symptoms in schizophrenic patients maintained on antipsychotic drugs, excluding clozapine.⁹⁵ Drugs modulating GABA activity can differentially affect working memory performance and brain function in schizophrenia.⁹⁶

Further clinical support for the relevance of GABA function for negative symptoms and cognitive functions comes from a study examining the relationship between visuomotor performance, working memory and negative symptoms. GABA neurotransmission within the dorso-lateral prefrontal cortex is critical for normal working memory function,^{97,98} goal maintenance, and interference control.⁹⁹ In a series of studies examining the relationships between diverse cognitive processes and functions, Silver et al.^{100,101} reported a strong association between the ability to resist distraction on a visuomotor task and verbal working memory in schizophrenia patients and interpreted this as reflecting shared prefrontal

cortex attention mechanisms. The ability to resist distraction in the visuomotor task was also associated with negative (but not positive) symptoms,¹⁰¹ suggesting that negative symptoms also share these attention resources. Given the important role of GABA in goal maintenance and interference control, these findings support the view that GABA plays a functional role in negative and cognitive symptoms of schizophrenia. One prediction of our hypothesis then is that GABA system modification by SSRI–antipsychotic combination may improve cognitive impairment in schizophrenia. This is currently under investigation.

CLINICAL IMPLICATIONS

Accumulating evidence supports the view that SSRI augmentation of antipsychotics is a useful treatment addition, and that studying its mechanism can serve as a window to understanding the mechanisms of synergism in schizophrenia. The findings in animal studies that SSRI–antipsychotic combinations produced unique effects support the view that specific pathways are modulated, and the findings that modulation involves the GABA system are consistent with clinical findings. Evidence that patients treated with SSRI–antipsychotic combination show changes in peripheral mononuclear cells consistent with those of animal studies and include proteins that are found in the brain (e.g., neurogranin) is encouraging and provides some, albeit limited, indication that the laboratory findings may have clinical relevance. Clearly, the preliminary nature of the data must be emphasized and many questions remain to be addressed in future studies. At the molecular level it will be necessary to identify the precise sequences by which the drugs interact to alter PKC and other GABA modulators, and how they interact with other neurotransmitter and cell signaling systems that also show unique responses to the combination treatment.

At the clinical level, studies will need to determine, among other questions, whether the mechanisms relevant for negative symptom amelioration also apply to other conditions that respond to SSRI–antipsychotic combination, such as resistant obsessive-compulsive disorders and depression, and whether particular combinations of SSRIs and antipsychotics differ in efficacy or show selectivity for particular clinical symptom profiles.

FINAL COMMENTS AND FUTURE PERSPECTIVES

Negative symptoms in schizophrenia, like other treatment-resistant symptoms of psychiatric illnesses, continue to be therapeutic challenges despite several decades of new drug development. Clearly, new paradigms and new molecular targets are needed to develop novel and

more effective pharmacotherapeutic compounds. Given the complexity of the conditions, it is unlikely that single-action drugs given alone will be effective, and therefore a multifunctional approach, as in augmentation treatment, may be needed.

We have argued that the SSRI–antipsychotic combination provides a novel paradigm that can advance understanding of the multifunctional mechanisms responsible for complex clinical phenotypes. The studies described here support the view that SSRI–antipsychotic combination produces unique molecular changes in the brain, changes that differ from those seen with the individual drugs. These changes are localized anatomically, and involve systems at various cellular levels, downstream from the initial impact of the drugs on membrane receptors or transporters. They modify widespread pathways and cellular processes, such as rate of neurotransmission, connectivity, plasticity, and proliferation.

We have highlighted some of the systems which current evidence suggests may provide plausible molecular meeting points for actions of pharmacologically distinct treatments and may underlie their convergent clinical effects. Clearly, the available data are limited, and much research is needed to identify and validate the molecular targets of SSRI–antipsychotic and other augmentation treatments. Conceptually, the method of isolating molecular effects of pharmacologically distinct but clinically convergent drugs provides a useful window for an understanding of the mechanisms of multifunctional treatments and development of new more effective drugs.

REFERENCES

1. Andreasen NC, Nopoulos P, Schultz S, et al. Positive and negative symptoms of schizophrenia: past, present, and future. *Acta Psychiatr Scand Suppl* 1994;384:51–59.
2. Berman RM, Narasimhan M, Charney DS. Treatment-refractory depression: definitions and characteristics. *Depress Anxiety* 1997; 5:154–164.
3. Dell'Osso B, Altamura AC, Mundo E, Marazziti D, Hollander E. Diagnosis and treatment of obsessive-compulsive disorder and related disorders. *Int J Clin Pract* 2007;61:98–104.
4. Lieberman JA, Stroup TS, McEvoy JP, et al.; Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) Investigators. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N Engl J Med* 2005;353:12:1209–1223.
5. Lewis SW, Barnes TRE, Davies L, et al. Randomized controlled trial of effect of prescription of clozapine versus other second-generation antipsychotic drugs in resistant schizophrenia. *Schizophr Bull* 2006;32:4:715–723.
6. Fava M. Augmentation and combination strategies in treatment-resistant depression. *J Clin Psychiatry* 2001;62 Suppl 18:4–11.
7. Silver H. Fluvoxamine as an adjunctive agent in schizophrenia. *CNS Drug Rev* 2001;7:283–304.
8. Burton S. Symptom domains of schizophrenia: the role of atypical antipsychotic agents. *J Psychopharmacol* 2006;20:6–19.
9. Rummel C, Kissling W, Leucht S. Antidepressants for the negative symptoms of schizophrenia. *Cochrane Database Syst Rev* 2006;3:CD005581.
10. Silver H, Barash I, Aharon N, Kaplan A, Poyurovsky M. Fluvoxamine augmentation of antipsychotics improves negative symptoms in psychotic chronic schizophrenic patients: a placebo-controlled study. *Int Clin Psychopharmacol* 2000;15:257–261.

11. Silver H, Nassar A. Fluvoxamine improves negative symptoms in treated chronic schizophrenia: an add-on double-blind, placebo-controlled study. *Biol Psychiatry* 1992;31:698–704.
12. Silver H, Shmugliakov N. Augmentation with fluvoxamine but not maprotiline improves negative symptoms in treated schizophrenia: evidence for a specific serotonergic effect from a double-blind study. *J Clin Psychopharmacol* 1998;8:208–211.
13. Chaichan W. Olanzapine plus fluvoxamine and olanzapine alone for the treatment of an acute exacerbation of schizophrenia. *Psychiatry Clin Neurosci* 2004;58:364–368.
14. Hiemke C, Peled A, Jabarin M, et al. Fluvoxamine augmentation of olanzapine in chronic schizophrenia: pharmacokinetic interactions and clinical effects. *J Clin Psychopharmacol* 2002;22:502–506.
15. Lammers CH, Deuschle M, et al. Coadministration of clozapine and fluvoxamine in psychotic patients: clinical experience. *Pharmacopsychiatry* 1999;32:76–77.
16. Silver H, Kaplan A, Jahjah N. Fluvoxamine augmentation for clozapine-resistant schizophrenia. *Am J Psychiatry* 1995;152:1098.
17. Silver H, Kushnir M, Kaplan A. Fluvoxamine augmentation in clozapine-resistant schizophrenia: an open pilot study. *Biol Psychiatry* 1996;40:671–674.
18. Szegei A, Anghelescu I, Wiesner J, et al. Addition of low-dose fluvoxamine to low-dose clozapine monotherapy in schizophrenia: drug monitoring and tolerability data from a prospective clinical trial. *Pharmacopsychiatry* 1999;32:148–153.
19. Silver H, Aharon N, Kaplan A. Add-on fluvoxamine improves primary negative symptoms: evidence for specificity from response analysis of individual symptoms. *Schizophr Bull* 2003;29:541–546.
20. Silver H, Nassar A, Aharon N, Kaplan A. The onset and time course of response of negative symptoms to add-on fluvoxamine treatment. *Int Clin Psychopharmacol* 2003;18:87–92.
21. Brøsen K. Differences in interactions of SSRIs. *Int Clin Psychopharmacol* 1998;13 Suppl 5:S45–S47.
22. Spina E, de Leon J. Metabolic drug interactions with newer antipsychotics: a comparative review. *Basic Clin Pharmacol Toxicol* 2007;100:4–22.
23. Sproule BA, Otton SV, Cheung SW, Zhong XH, Romach MK, Sellers EM. CYP2D6 inhibition in patients treated with sertraline. *J Clin Psychopharmacol* 1997;17:102–106.
24. Weigmann H, Gerek S, Zeisig A, Müller M, Härtter S, Hiemke C. Fluvoxamine but not sertraline inhibits the metabolism of olanzapine: evidence from a therapeutic drug monitoring service. *Ther Drug Monit* 2001;23:410–413.
25. Wetzell H, Anghelescu I, Szegei A, et al. Pharmacokinetic interactions of clozapine with selective serotonin reuptake inhibitors: differential effects of fluvoxamine and paroxetine in a prospective study. *J Clin Psychopharmacol* 1998;18:2–9.
26. Avenoso A, Spina E, Campo G, et al. Interaction between fluoxetine and haloperidol: pharmacokinetic and clinical implications. *Pharmacol Res* 1997;35:335–339.
27. Yasui-Furukori N, Kondo T, Mihara K, Inoue Y, Kaneko S. Fluvoxamine dose-dependent interaction with haloperidol and the effects on negative symptoms in schizophrenia. *Psychopharmacology (Berl)* 2004;171:223–227.
28. Baldessarini RJ, Cohen BM, Teicher MH. Significance of neuroleptic dose and plasma level in the pharmacological treatment of psychoses. *Arch Gen Psychiatry* 1988;45:79–91.
29. de Oliveira IR, Dardennes RM, Amorim ES, et al. Is there a relationship between antipsychotic blood levels and their clinical efficacy? An analysis of studies design and methodology. *Fundam Clin Pharmacol* 1995;9:488–502.
30. Volavka J, Cooper TB, Czobor P, et al. High-dose treatment with haloperidol: the effect of dose reduction. *J Clin Psychopharmacol* 2000;20:252–256.
31. Poyurovsky M, Kurs R, Weizman A. Olanzapine-sertraline combination in schizophrenia with obsessive-compulsive disorder. *J Clin Psychiatry* 2003;64:611.
32. Goff DC, Freudenreich O, Evins AE. Augmentation strategies in the treatment of schizophrenia. *CNS Spectr* 2001;6:904,907–911.
33. Stoll AL, Haura G. Tranylcypromine plus risperidone for treatment-refractory major depression. *J Clin Psychopharmacol* 2000;20:495–496.
34. Tsai G, Coyle JT. Glutamatergic mechanisms in schizophrenia. *Annu Rev Pharmacol Toxicol* 2002;42:165–179.
35. Siris SG. Adjunctive medication in the maintenance treatment of schizophrenia and its conceptual implications. *Br J Psychiatry Suppl* 1993;(22):66–78.
36. Silver H, David D, Kaplan M, et al. An examination of the factor structure of schizophrenic symptoms and comparison of different rating scales. *Schizophr Res* 1993;10:67–75.
37. Silver H. Selective serotonin reuptake inhibitor augmentation in the treatment of negative symptoms of schizophrenia. *Int Clin Psychopharmacol* 2003;18:305–313.
38. Chertkow Y, Weinreb O, Youdim MB, Silver H. Dopamine and serotonin metabolism in response to chronic administration of fluvoxamine and haloperidol combined [Erratum in: *J Neural Transm* 2007;114:1443–1454]. *J Neural Transm* 2007;114:1455.
39. Rapoport M, van Reekum R, Mayberg H. The role of the cerebellum in cognition and behavior: a selective review. *J Neuropsychiatry Clin Neurosci* 2000;12:193–198.
40. Wong DT, Bymaster FP, Reid LR, Threlkeld PG. Fluoxetine and two other serotonin uptake inhibitors without affinity for neuronal receptors. *Biochem Pharmacol* 1983;32:1287–1293.
41. Silver H, Youdim MB. MAO-A and MAO-B activities in rat striatum, frontal cortex and liver are unaltered after long-term treatment with fluvoxamine and desipramine. *Eur Neuropsychopharmacol* 2000;10:125–128.
42. Baldessarini RJ, Marsh ER, Kula NS. Interactions of fluoxetine with metabolism of dopamine and serotonin in rat brain regions. *Brain Res* 1992;579:152–156.
43. Moret C, Briley M. Effect of antidepressant drugs on monoamine synthesis in brain in vivo. *Neuropharmacology* 1992;31:679–684.
44. Penttilä J, Kajander J, Aalto S, et al. Effects of fluoxetine on dopamine D₂ receptors in the human brain: a positron emission tomography study with [¹¹C]raclopride. *Int J Neuropsychopharmacol* 2004;7:431–439.
45. Zhang W, Perry KW, Wong DT, et al. Synergistic effects of olanzapine and other antipsychotic agents in combination with fluoxetine on norepinephrine and dopamine release in rat prefrontal cortex. *Neuropsychopharmacology* 2000;23:250–262.
46. Denys D, Klompmaers AA, Westenberg HG. Synergistic dopamine increase in the rat prefrontal cortex with the combination of quetiapine and fluvoxamine. *Psychopharmacology (Berl)* 2004;176:195–203.
47. Koch S, Perry KW, Bymaster FP. Brain region and dose effects of an olanzapine-fluoxetine combination on extracellular monoamine concentrations in the rat. *Neuropharmacology* 2004;46:232–242.
48. Ago Y, Nakamura S, Baba A, Matsuda T. Sulpiride in combination with fluvoxamine increases in vivo dopamine release selectively in rat prefrontal cortex. *Neuropsychopharmacology* 2005;30:43–51.
49. Waldmeier PC. Analysis of the activation of dopamine metabolism by a serotonin uptake inhibitor. *Eur J Pharmacol* 1979;60:315–322.
50. Waldmeier PC, Delini-Stula AA. Serotonin-dopamine interactions in the nigrostriatal system. *Eur J Pharmacol* 1979;55:363–373.
51. Chertkow Y, Weinreb O, Youdim MB, Silver H. The effect of chronic co-administration of fluvoxamine and haloperidol compared to clozapine on the GABA system in the rat frontal cortex. *Int J Neuropsychopharmacol* 2006;9:287–296.
52. Danovich L, Weinreb O, Youdim MB, Silver H. The molecular mechanism of pharmacodynamic interactions between psychoactive drugs: interactions between antidepressants and antipsychotic drugs. Presented at: Israel Society for Neuroscience Conference Eilat 2007; November 25–27, 2007; Eilat, Israel.
53. Kronfol Z, Remick DG. Cytokines and the brain: implications for clinical psychiatry. *Am J Psychiatry* 2000;157:683–94.

54. Tian J, Chau C, Hales TG, Kaufman DL. GABA_A receptors mediate inhibition of T cell responses. *J Neuroimmunol* 1999;96: 21–28.
55. Avissar S, Roitman G, Schreiber G. Differential effects of the antipsychotics haloperidol and clozapine on G protein measures in mononuclear leukocytes of patients with schizophrenia. *Cell Mol Neurobiol* 2001;21:799–811.
56. Ilani T, Ben-Shachar D, Strous RD, et al. A peripheral marker for schizophrenia: increased levels of D₃ dopamine receptor mRNA in blood lymphocytes. *Proc Natl Acad Sci U S A* 2001;98:625–628.
57. Gladkevich A, Kauffman HF, Korf J. Lymphocytes as a neural probe: potential for studying psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 2004;28:559–576.
58. Tang Y, Gilbert DL, Glauser TA, Hershey AD, Sharp FR. Blood gene expression profiling of neurologic diseases: a pilot microarray study. *Arch Neurol* 2005;62:210–215.
59. Bowden NA, Weidenhofer J, Scott RJ, et al. Preliminary investigation of gene expression profiles in peripheral blood lymphocytes in schizophrenia. *Schizophr Res* 2006;82:175–183.
60. Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA. The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. *J Lab Clin Med* 2006;147:126–132.
61. Rothermundt M, Arolt V, Bayer TA. Review of immunological and immunopathological findings in schizophrenia. *Brain Behav Immun* 2001;15:319–339.
62. Chertkow Y, Weinreb O, Youdim MB, Silver H. Gene expression changes in peripheral mononuclear cells from schizophrenic patients treated with a combination of antipsychotic with flvoxamine. *Prog Neuropsychopharmacol Biol Psychiatry* 2007;31: 1356–1362.
63. Dean B, Hussain T, Hayes W, et al. Changes in serotonin_{2A} and GABA_A receptors in schizophrenia: studies on the human dorso-lateral prefrontal cortex. *J Neurochem* 1999;72:1593–1599.
64. Toyooka K, Watanabe Y, Iritani S, et al. A decrease in interleukin-1 receptor antagonist expression in the prefrontal cortex of schizophrenic patients. *Neurosci Res* 2003;46:299–307.
65. Mirnics K, Middleton FA, Stanwood GD, Lewis DA, Levitt P. Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia. *Mol Psychiatry* 2001;6:293–301.
66. Bowden NA, Scott RJ, Tooney PA. Altered expression of regulator of G-protein signalling 4 (RGS4) mRNA in the superior temporal gyrus in schizophrenia. *Schizophr Res* 2007;89:165–168.
67. Broadbelt K, Ramprasad A, Jones LB. Evidence of altered neuropeptide immunoreactivity in areas 9 and 32 of schizophrenic prefrontal cortex. *Schizophr Res* 2006;87:6–14.
68. Reynolds GP, Zhang ZJ, Beasley CL. Neurochemical correlates of cortical GABAergic deficits in schizophrenia: selective losses of calcium binding protein immunoreactivity. *Brain Res Bull* 2001;55:579–584.
69. Blum BP, Mann JJ. The GABAergic system in schizophrenia. *Int J Neuropsychopharmacol* 2002;5:159–179.
70. Hashimoto T, Bazmi HH, Mirnics K, Wu Q, Sampson AR, Lewis DA. Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. *Am J Psychiatry* 2008;165:479–489.
71. Kalkman HO, Loetscher E. GAD₆₇: the link between the GABA-deficit hypothesis and the dopaminergic- and glutamatergic theories of psychosis. *J Neural Transm* 2003;110:803–812.
72. Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA. Decreased glutamic acid decarboxylase₆₇ messenger RNA expression in a subset of prefrontal cortical γ -aminobutyric acid neurons in subjects with schizophrenia. *Arch Gen Psychiatry* 2000;57: 237–245.
73. Benes FM, Vincent SL, Marie A, Khan Y. Up-regulation of GABA_A receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. *Neuroscience* 1996;75:1021–1031.
74. Deng C, Huang XF. Increased density of GABA_A receptors in the superior temporal gyrus in schizophrenia. *Exp Brain Res* 2006; 168:587–590.
75. Javitt DC. Is the glycine site half saturated or half unsaturated? Effects of glutamatergic drugs in schizophrenia patients. *Curr Opin Psychiatry* 2006;19:151–157.
76. Shim SS, Hammonds MD, Kee BS. Potentiation of the NMDA receptor in the treatment of schizophrenia: focused on the glycine site. *Eur Arch Psychiatry Clin Neurosci* 2008;258:16–27.
77. Konradi C, Heckers S. Molecular aspects of glutamate dysregulation: implications for schizophrenia and its treatment. *Pharmacol Ther* 2003;97:153–79.
78. Weinberger DR, Berman KF. Prefrontal function in schizophrenia: confounds and controversies. *Philos Trans R Soc Lond B Biol Sci* 1996;351:1495–503.
79. Daskalakis ZJ, Fitzgerald PB, Christensen BK. The role of cortical inhibition in the pathophysiology and treatment of schizophrenia. *Brain Res Rev* 2007;56:427–42.
80. Eggen SM, Hashimoto T, Lewis DA. Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. *Arch Gen Psychiatry* 2008;65:772–784.
81. Silver H, Feldman P, Bilker W, Gur RC. Working memory deficit as a core neuropsychological dysfunction in schizophrenia. *Am J Psychiatry* 2003;160:1809–1816.
82. Schmitt A, May B, Müller B, et al. Effects of chronic haloperidol and clozapine treatment on AMPA and kainate receptor binding in rat brain. *Pharmacopsychiatry* 2003;36:292–296.
83. Zhang HX, Zhao JP, Lv LX, et al. Explorative study on the expression of neuregulin-1 gene in peripheral blood of schizophrenia. *Neurosci Lett* 2008;438:1–5.
84. Fumagalli F, Frasca A, Racagni G, Riva MA. Dynamic regulation of glutamatergic postsynaptic activity in rat prefrontal cortex by repeated administration of antipsychotic drugs. *Mol Pharmacol* 2008;73:1484–1490.
85. Tarazi FI, Baldessarini RJ, Kula NS, Zhang K. Long-term effects of olanzapine, risperidone, and quetiapine on ionotropic glutamate receptor types: implications for antipsychotic drug treatment. *J Pharmacol Exp Ther* 2003;306:1145–1151.
86. Zink M, Schmitt A, May B, et al. Differential effects of long-term treatment with clozapine or haloperidol on GABA_A receptor binding and GAD67 expression. *Schizophr Res* 2004;66:151–157.
87. Zink M, Schmitt A, May B, Müller B, Braus DF, Henn FA. Differential effects of long-term treatment with clozapine or haloperidol on GABA transporter expression. *Pharmacopsychiatry* 2004;37:171–174.
88. Zink M, Schmitt A, May B, et al. Differential effects of long-term treatment with clozapine or haloperidol on GABA_A receptor binding and GAD67 expression. *Schizophr Res* 2004;66:151–157.
89. Feng J, Cai X, Zhao J, Yan Z. Serotonin receptors modulate GABA_A receptor channels through activation of anchored protein kinase C in prefrontal cortical neurons. *J Neurosci* 2001;21:6502–6511.
90. Pinna G, Costa E, Guidotti A. Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteroid content at doses that are inactive on 5-HT reuptake. *Psychopharmacology (Berl)* 2006;186:362–372.
91. Silver H, Knoll G, Isakov V, Goodman C, Finkelstein Y. Blood DHEAS concentrations correlate with cognitive function in chronic schizophrenia patients: a pilot study. *J Psychiatr Res* 2005;39:569–575.
92. Strous RD. Dehydroepiandrosterone (DHEA) augmentation in the management of schizophrenia symptomatology. *Essent Psychopharmacol* 2005;6:141–147.
93. Tsai GE, Yang P, Chang YC, Chong MY. D-Alanine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* 2006;59:230–234.
94. Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Silipo G, Lichtenstein M. Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. *Arch Gen Psychiatry* 1999;56:29–36.
95. Lane HY, Huang CL, Wu PL, et al. Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to clozapine for the treatment of schizophrenia. *Biol Psychiatry* 2006;60:645–9.
96. Menzies L, Ooi C, Kamath S, et al. Effects of aminobutyric

- acid-modulating drugs on working memory and brain function in patients with schizophrenia. *Arch Gen Psychiatry* 2007;64:156–167.
97. Sawaguchi T, Matsumura M, Kubota K. Delayed response deficit in monkeys by locally disturbed prefrontal neuronal activity by bicuculline. *Behav Brain Res* 1988;31:193–198.
98. Rao SG, Williams GV, Goldman-Rakic PS. Destruction and creation of spatial tuning by disinhibition: GABA_A blockade of prefrontal cortical neurons engaged by working memory. *J Neurosci* 2000;20:485–494.
99. Lewis DA, Volk DW, Hashimoto T. Selective alterations in prefrontal cortical GABA neurotransmission in schizophrenia: a novel target for the treatment of working memory dysfunction. *Psychopharmacology* 2004;174;1:143–150.
100. Silver H, Shlomo N, Schwartz M, Hocherman S. Impaired visuo-motor function in schizophrenic patients compared with control subjects. *J Neuropsychiatry Clin Neurosci* 2002;14:72–76.
101. Silver H, Feldman P. Evidence for sustained attention and working memory in schizophrenia sharing a common mechanism. *J Neuropsychiatry Clin Neurosci* 2005;17:391–398.