

**Time Dependent Cognitive Deficits Associated with First and Second Generation
Antipsychotics: Cholinergic Dysregulation as a Potential Mechanism**

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Abbreviations:

AChE, acetylcholinesterase
BDNF, Brain Derived Growth Factor
ChAT, choline acetyltransferase
CPZ, chlorpromazine
Enzyme Linked Immunosorbent Assay, ELISA
FGA, first generation antipsychotic
HAL, haloperidol
Long Term Potentiation, LTP
Muscarinic acetylcholine receptor, mAChR
NGF, Nerve Growth Factor
Nicotinic acetylcholine receptor, nAChR
OLZ, olanzapine
RISP, risperidone
SGA, second generation antipsychotic
Tardive Dyskinesia, TD
TrkA, tropomyosin-receptor kinase A
VAcHT, vesicular acetylcholine transporter

Abstract

While cognitive dysfunction is considered one of the more debilitating symptoms of schizophrenia, there is a fundamental gap in our knowledge of how the primary pharmacologic treatments of this disease, first and second generation antipsychotics (FGAs and SGAs, respectively) affect cognition, particularly over extended periods of time. Moreover, it has been known for decades that chronic treatment with FGAs can lead to imbalances in cholinergic function in the striatum that result in movement disorders, however, there is a growing body of evidence to suggest that both FGAs and SGAs can lead to cholinergic alterations in brain areas more traditionally considered as memory-related, such as cortical and hippocampal regions. Data from our laboratories in rodents indicate that some SGAs (if administered for sufficient periods of time) can be associated with impairments in memory-related task performance as well as alterations in the cholinergic enzyme, choline acetyltransferase, the vesicular acetylcholine transporter, nicotinic (α_7) and muscarinic (M_2) acetylcholine receptors. Given the well-documented importance of central cholinergic function to information processing and cognitive function, it is important that the mechanisms for such chronic antipsychotic effects be identified. In this review, two potential mechanisms for long term antipsychotic-related cholinergic alterations in the CNS are discussed: 1) antipsychotic antagonist activity at dopaminergic- D_2 receptors on cholinergic neurons and 2) antipsychotic effects on neurotrophins that support cholinergic neurons such as nerve growth factor (NGF) and brain derived growth factor (BDNF). Novel strategies to optimize the therapeutics of schizophrenia and maintain cognitive function via adjunctive cholinergic compounds and antipsychotic crossover approaches are also discussed.

Introduction

Schizophrenia is a chronic and disabling illness characterized by severe behavioral symptoms that commonly require life-long therapeutic intervention. Fortunately, the pharmacologic treatments for schizophrenia which include first and second generation antipsychotics (FGAs and SGAs, respectively) have been demonstrated in numerous clinical trials to improve the behavioral symptoms in most schizophrenic patients. It has been widely held since their advent in the late 1980s, that SGAs (as a class of compounds) have many advantages over FGAs including greater improvements in negative symptoms, prevention of relapse, increased functional capacity, fewer movement-related side effects, and superior effects on cognition (reviewed, Miyamoto et al., 2005). Accordingly, SGAs presently account for nearly all of the new prescriptions written for neuroleptics in the United States. While only specifically FDA-approved for treating psychotic symptoms in schizophrenia and bipolar disease, SGAs are now routinely used “off-label” for the behavioral symptoms of other illnesses in patients, ranging in age from the very young (e.g., in autism and ADHD), to the very old (e.g., Alzheimer’s disease). This widespread “off-label” drug use is beginning to cause controversy now that several long term side effects have emerged with SGAs such as abnormal weight gain, development of diabetes mellitus, hyperlipidemias, etc. (reviewed, Gardner et al., 2005). Further concern has been fueled by the first phase of results (Lieberman et al., 2005) of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE). In this large prospective clinical study, the SGAs, olanzapine, quetiapine, risperidone, and ziprasidone appeared to offer few significant advantages over the FGA, perphenazine, when dropout rates (ostensibly due to intolerable side effects, lack of efficacy, etc.) were considered. Given the well-documented challenges associated with noncompliance to therapeutic regimens in schizophrenia, there could be several possible

interpretations of the CATIE study, however, collectively; the information summarized above would strongly support the conclusion that a more critical look at SGAs is warranted, particularly since they are markedly more expensive than conventional antipsychotics.

Antipsychotics and Cognitive Function

If SGAs (compared to FGAs) indeed have superior effects on cognition as has been reported (see Keefe et al., 2006), then the continued preference for SGAs in the treatment of schizophrenia would be warranted. Neuropsychological evaluations of schizophrenic patients consistently indicate cognitive impairments approaching two standard deviations below the mean of control subjects (Wilk et al., 2004). Such deficits are evident at the onset of the illness and in contrast to the positive and negative symptoms (which tend to be episodic), cognitive decrements in schizophrenia generally persist throughout the illness (Nuechterlein et al., 1992). In addition, compared to other symptom domains, cognitive dysfunction in schizophrenia (particularly in the domains of memory, executive function, and vigilance) appears to have the greatest impact on key indicators of functional outcome which include the acquisition of social skills, success in rehabilitation programs, and vocational achievements (reviewed, Green et al., 2000).

As noted above, studies focused on the cognitive effects of antipsychotics have commonly reported superior effects of SGAs over FGAs and several studies have further suggested that SGAs improve cognitive function (reviewed, Harvey et al., 2004). However, a parsimonious interpretation of the currently available literature on this subject would have to take into consideration methodological weaknesses and other limitations of many of the studies. For example, in earlier studies, confounding variables included relatively wide study group heterogeneity, cognitive testing while florid psychotic symptoms were present, and extensive prior antipsychotic exposure by the study subjects. Other limitations included polypharmacy at

the time of cognitive testing and the concomitant use of anticholinergic drugs (i.e., drugs known to impair cognition) by those treated with FGAs to control extrapyramidal symptoms (Sharma and Mockler, 1998). A common limitation of both older and newer studies is the large, potentially inappropriate doses of the FGAs used in the comparisons (Carpenter and Gold, 2002). While some of the aforementioned study design flaws have been addressed in more recent investigations, many of the newer studies have been retrospective or open label in design and virtually all the studies have been of relatively short duration (i.e., they have rarely exceeded a few months to a year in length, even though most schizophrenic patients require decades of antipsychotic therapy).

While there are a few exceptions (e.g., Wolff and Leander, 2003), the results of most animal studies conducted to date suggest that SGAs and FGAs are either inactive, or that they exert negative effects on cognition. In acute studies, the FGA, haloperidol (Ploeger et al. 1992), as well as the SGAs, clozapine, olanzapine, and risperidone impaired place navigation in the Morris Water Maze (Skarsfeldt, 1996). Haloperidol (Beatty and Rush, 1983), clozapine (Addy and Levin 2002), and olanzapine (Levin et al., 2005) impaired spatial working memory in radial arm maze tests, and haloperidol, clozapine and risperidone impaired performance of a delayed non-match to position (DNMTP) task (Didriksen, 1995). In chronic studies (which are much more relevant to the more common clinical use of antipsychotics in schizophrenia), haloperidol, clozapine, and risperidone impaired acquisition in a radial arm maze task in rats while olanzapine had no measurable adverse effects (Rosengarten and Quartermain, 2002). In another chronic study, haloperidol was found to disrupt radial arm maze choice accuracy in rats, but only during the first week of administration (Levin et al., 1987). Most recently, Didriksen and colleagues (Didriksen et al., 2006) found that acute administration of clozapine and olanzapine

impaired water maze performance in rats (replicating earlier studies), while in chronically treated animals, the impairments abated in the clozapine treated animals, but were exacerbated in the olanzapine-treated animals. Interestingly, the SGA sertindole remained without effect on water maze performance following acute or chronic treatment in these studies.

The results of studies in our laboratories to date also indicate that like FGAs, representative SGAs (e.g., olanzapine, risperidone, ziprasidone) can impair some cognitive tasks if they are administered for sufficiently long periods of time. Fig 1 provides a summary of six different chronic studies conducted in our laboratory that support such a conclusion. Rats were administered either vehicle alone (very dilute acetic acid in distilled water), haloperidol 2.0, risperidone 2.5, chlorpromazine 10.0, or olanzapine 10.0 mg/kg/day in drinking water for periods ranging from 45 to 180 days. After the treatment period, they were given a drug-free washout period and then tested in a water maze task. In order to assess the effects of the length of time of drug administration across the studies, the area under the latency learning curve for each animal was calculated and then group performances were compared across the studies statistically (two-way ANOVA for effects of treatment and time of administration). This method of comparing latency area under the curve (AUC) in water maze studies to provide a more simple comparison between groups has been published previously (Youngblood et al., 1997). As shown in Fig 1, there were no detectable antipsychotic effects on AUC at the 45 day time period, however, by 90 days of treatment, haloperidol, risperidone, and olanzapine, were associated with impairments that were also present (or a significant trend toward impairment was observed) at the 180 day time period. It is also important to note, that under vehicle conditions, those treated for 180 days were impaired when compared to the 45 day time point. The basis for this finding is unclear but could reflect an age-related effect since the rats were approximately 9-10 months old by the 180

day time point. We have observed (for example) some evidence of water maze learning impairments in 12 month old rats in previous studies. The chlorpromazine data for the 90 and 180 day time points are included in the figure (for illustration purposes), but were not included in the statistical analysis since we have not yet conducted a 45 day study with this compound.

Collectively, when interpreting the studies described in the preceding paragraphs there are some limitations that should be considered. For example, in several of the studies, there was no clear justification for the doses selected or the method of drug delivery used and, therefore, logical questions regarding clinical relevance of the results arise (see Kapur et al., 2003). In addition, the experiments described above (and most studies to date) were conducted in normal animals (i.e., not in animal models derived to express symptoms of schizophrenia or other psychiatric disorders). In the studies conducted in our laboratories (e.g., Terry et al., 2005a), dosing parameters such as plasma antipsychotic levels achieved and predicted D₂ occupancy values were considered. Furthermore, given the wide use of antipsychotics in conditions as diverse as ADHD, autism, schizophrenia, and Alzheimer's disease, conducting experiments in normal animals is a logical initial approach to understanding chronic antipsychotic effects on the mammalian brain that could be relevant to a variety of diseases. Nevertheless, a comprehensive (and therapeutically-relevant) understanding of the effects of chronic antipsychotic treatments on cognitive function will require experiments in animal models of psychiatric illness.

Antipsychotics and the Central Cholinergic System

While the therapeutic agents used to treat schizophrenia should ideally target the actual pathophysiological causes of the illness, to date, such mechanisms have not been unequivocally established. Unfortunately, this lack of knowledge applies to the underlying neurobiological substrates of the behavioral symptoms as well as the cognitive deficits in schizophrenia.

Abnormalities in the neurotransmitters dopamine, serotonin, and glutamate have commonly been implicated in many aspects of the neuropathology of schizophrenia, however, there is some (albeit limited) evidence that alterations in acetylcholine neurotransmission are present as well (see Sarter et al., 2005). Such data may be especially significant to the cognitive deficits, since cholinergic neurons, particularly those originating in the basal forebrain, influence many components of information processing, including attention, encoding, memory consolidation, and retrieval (see review, Gold 2003). The evidence includes significant correlations between reduced choline acetyltransferase (i.e., ChAT, the acetylcholine synthesizing enzyme) levels and impaired cognition in schizophrenia, decreases in both nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine receptors (mAChRs) in postmortem brains of schizophrenics, the later finding has been verified by single photon emission computed tomography (SPECT) in living, unmediated schizophrenic patients (see review, Friedman, 2004).

In light of the information provided above, an interesting question arises as to what extent antipsychotics affect the central cholinergic system. The FGAs, thioridazine and chlorpromazine, and the SGAs, clozapine and olanzapine, have been shown to bind all of the known muscarinic acetylcholine receptor subtypes with relatively high affinity (reviewed, Bymaster et al., 2003), although the significance of this binding to the therapeutics and adverse effects is unknown. Interestingly, while both FGAs and SGAs have been observed to increase acetylcholine levels in the hippocampus of rats (Shirazi-Southhall et al., 2002), the effects of the SGAs, clozapine and olanzapine, were considerably more robust. Further, the SGAs, clozapine, olanzapine, risperidone, and ziprasidone significantly increased acetylcholine release in rat medial prefrontal cortex, whereas the FGAs, haloperidol and thioridazine did not (Ichikawa et al., 2002). Such differential effects on acetylcholine release were hypothesized (in the studies

cited above) to possibly underlie the reported superiority of SGAs on cognitive function when compared to FGAs. However, given that these studies evaluated acute drug effects on basal efflux of acetylcholine in the brains of animals that were not behaviorally tested, several questions are left unanswered such as 1) whether such effects would persist for extended periods of time (i.e., the more clinically relevant question regarding antipsychotics) and, 2. How such antipsychotic effects on basal release of acetylcholine might be expected to influence cognitive processes that may require periodic fluctuations (i.e., increases or decreases) in cholinergic activity.

To date, most of the data regarding the chronic effects of antipsychotic drugs on central cholinergic neurons have come from studies designed to investigate the basis of their adverse motor reactions. For example, an increase in the activity of cholinergic interneurons in the striatum in response to FGAs has been reported to initially parallel adverse extrapyramidal effects in humans, whereas longer treatment periods have been associated with decreases in cholinergic activity below baseline (i.e., effects that correspond with the emergence of tardive dyskinesia (TD), see Miller and Chouinard, 2003). Such temporal effects may account for the ability of anticholinergic drugs (e.g., benztropine) to ameliorate extrapyramidal side effects of FGAs during early use, and their lack of efficacy (or even tendency to exacerbate symptoms) in TD. While SGAs are commonly reported to be associated with fewer extrapyramidal symptoms and a lower risk of TD than FGAs, such reports should not be construed to suggest that SGAs are free of these adverse effects. In fact, a brief perusal of the manufacturers package insert will clearly indicate that such adverse effects can be associated with virtually any of these agents with the possible exception of clozapine. While the currently available evidence also suggests that the risks of TD are probably lower with most SGAs when compared with FGAs of high potency, this

may not necessarily be the case with FGAs of low potency (e.g., chlorpromazine) taken at moderate doses (Gardner et al., 2005).

Given the evidence of a possible cholinergic basis for antipsychotic related extrapyramidal effects and TD, another logical question arises as to whether such cholinergic effects might influence information processing and cognition as well, particularly since cholinergic interneurons in the striatum have been shown to exhibit long term potentiation (LTP, Suzuki et al., 2001) and to play an important role procedural learning (Kitabatake et al., 2003). In addition, several years ago the FGA haloperidol (administered chronically to rats) was observed to decrease ChAT immunoreactivity as well as ChAT enzyme activity in the hippocampus (Mahadik et al, 1988), a structure well known for its role in encoding, consolidation and episodic memory (Squire 1994). More recently, we have detected time-dependent effects of both representative FGAs and SGAs on ChAT, the vesicular acetylcholine transporter (VAcHT), as well as nicotinic (α_7) and muscarinic (M_2) acetylcholine receptors (Terry, et al., 2005, 2006a; 2006b) in other memory-related brain regions as well. Fig 2 provides a summary of some of these experiments where the cholinergic marker proteins, ChAT, and VAcHT, were quantified in rat brain after 90 days of treatment. The upper panel (A) of the figure illustrates immunostaining results, whereas, the lower panel (B) illustrates the effects of ELISA experiments that were conducted to measure levels of the cholinergic marker protein, VAcHT. Thus, 90 days of chronic treatment with several antipsychotics (i.e, both FGAs and SGAs) was associated with significant decreases in cholinergic marker proteins in the striatum as well as in brain regions more traditionally though to support cognition (e.g., basal forebrain, cortex).

Antipsychotics, Dopamine-Acetylcholine Interactions

The mechanism of the chronic effects of antipsychotic drugs on the levels of central cholinergic markers and cognitive function described above is presently unknown. All of the agents evaluated in the aforementioned experiments have significant antagonist activity at D₂ receptors and further, D₂ receptors have been localized on cell somata as well as axonal and dendritic processes of striatal cholinergic interneurons in the dorsal striatum and nucleus accumbens (Alcantara et al., 2003). These receptors are well known to inhibit acetylcholine release when bound by dopamine and in the context of TD, the D₂ receptor antagonist effects of FGAs in animals have been reported to result in excessive neuronal activity, intracellular accumulation of calcium, and subsequent cell damage (see Miller and Chouinard, 2003). It is interesting to hypothesize that such negative effects could affect mnemonic function given the reported role of cholinergic interneurons in the striatum in LTP and learning noted above. Further, our detection of sustained antipsychotic-related decreases in cholinergic markers in the cortex may reflect the involvement of dopamine-acetylcholine interactions in the nucleus accumbens. It has been hypothesized that dopamine in the nucleus accumbens inhibits the activity of GABAergic projections to the basal forebrain thus modulating the excitability of cholinergic neurons that project to the cortex (see Sarter and Bruno, 1999). Accordingly, chronic exposure to drugs that antagonize mesolimbic dopamine receptors could indirectly lead to imbalances in cholinergic activity in basal forebrain neurons (and thus projection areas such as the cortex). Our findings of antipsychotic-related decreases in cholinergic markers in the hippocampus may be even more difficult to interpret since D₂ receptors are relatively sparse in the septohippocampal pathway. However, it should be noted that dopamine regulation of septohippocampal cholinergic activity via the D₁ receptor has been described (Day and Fibiger,

1994) and several FGAs and SGAs (including those we evaluated) have significant antagonist activity at D₁ receptors (see Miyamoto et al., 2005).

Antipsychotics and Neurotrophins

The effects of antipsychotics on the neurotrophins, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) may be particularly germane to this review since both proteins are known to support cholinergic neurons in the adult mammalian brain and cognitive function (reviewed, Fahnstock et al., 2004; Counts and Mufson 2005). Interestingly, both acute (3 days) and subchronic (21 days) exposure to haloperidol in rats decreased the levels of BDNF in the prefrontal cortex, hippocampus, and amygdala. Decreased BDNF concentrations (Angelucci et al., 2000) and decreased expression of BDNF mRNA (Lipska et al., 2001) have also been observed after risperidone and clozapine administration. Another report indicated that subchronic (29 day) treatment with haloperidol or risperidone in rats increased NGF immunoreactivity in the hypothalamus, but decreased NGF levels in the striatum and hippocampus. The same investigators (Angelucci et al., 2005) more recently found that 29 days of oral olanzapine treatment increased NGF in the hippocampus, occipital cortex and hypothalamus, but decreased BDNF in the hippocampus and frontal cortex. The results of studies in our laboratories over the last several years indicate that the effects of both SGAs and FGAs on NGF are temporally dependent, specifically; they can be very different depending on the length of time of administration. Thus, whereas NGF levels in the hippocampus were either unchanged or upregulated in rats in response to several antipsychotics during short periods of treatment (7, 14, and 45 days) they were significantly decreased by haloperidol, chlorpromazine, olanzapine, and risperidone when continuously administered orally for 90 or 180 days (Pillai et al., 2006; Terry et al., 2006b). Fig. 3 provides a summary of some of the results in the

hippocampus obtained by our laboratories to date. In the upper portion of the figure (A) representative immunohistochemical images are provided, while in the lower portion of the figure (B), representative data from ELISA experiments illustrating the effects of several antipsychotics on NGF over time are provided. Thus, at the early time points (e.g., 45-days) SGAs did not appear to decrease NGF (and in the case of olanzapine even increased NGF substantially), whereas, after longer periods of treatment (90 days and beyond) all of the antipsychotics tested to date were associated with decreases in NGF in the hippocampus.

Notwithstanding the potentially important therapeutic ramifications of the studies cited above, the role of neurotrophins in brain function is a very complex, rapidly evolving, and poorly understood subject, and the study of neurotrophins in schizophrenia and other psychiatric disorders is in its infancy. The fields of neurodevelopment, aging, and Alzheimer's disease research clearly indicate that a more comprehensive understanding of such issues is likely to require many years of diligent research. Future studies in both normal and pathophysiologic states designed to characterize the distinct and possibly opposing actions of pro- and mature neurotrophins in regulating neuron survival and synaptic plasticity, as well efforts to further define the role of low and high affinity neurotrophin receptors (and their interactions) will be required.

Antipsychotics and Central Cholinergic Function: Therapeutic Implications

Given the reported cholinergic deficits in schizophrenia (described above), it has been hypothesized that a cholinergic strategy for ameliorating cognitive deficits using compounds such as acetylcholinesterase inhibitors (AChEIs), mAChR agonists, nAChR agonists, or allosteric potentiators of nAChR function might be viable (reviewed, Freedman 2004). To date, however, the only approach that has been evaluated clinically to any significant extent has been

the adjunctive administration of AChEIs with antipsychotics. The data from such studies have thus far been equivocal; the beneficial cognitive effects of donepezil or rivastigmine as add-on treatments to SGAs observed in preliminary open-label studies and case reports, were not confirmed in randomized, double-blind, and placebo controlled studies (see Friedman et al., 2002; Sharma et al., 2006). Most recently, a small, randomized, double-blind clinical trial (N=8) indicated that adjunctive treatment with the AChEI, galantamine, improved short-term memory and attention in schizophrenic or schizoaffective patients who were stabilized on risperidone (Schubert et al., 2006). While such data are certainly encouraging, much larger studies will be required to verify the validity of this approach as a reliable therapeutic intervention.

There may be several factors that underlie the equivocal nature of the studies noted above, such as cigarette smoking (and thus the confounding cholinergic receptor effects of nicotine) by the research subjects, exposure to other drugs of abuse, differences in the neuropsychological measures employed, etc. An important factor (not commonly discussed) that could certainly influence responses to the adjunctive agent is the unique medication history of the study subject. Specifically, factors such as the history of antipsychotic drug exposure, the particular antipsychotic agent currently being administered, and how long the patient has been exposed to this compound, could be especially important to the adjunctive treatment response. In our chronic animal studies, we have found that several antipsychotics (e.g. both FGAs and SGAs) can lead to decreases in cholinergic markers, as well as more specific effects on cholinergic receptors (e.g., decreases in α_7 nAChRs in association with risperidone and increases in M_2 mAChRs in association with olanzapine). Interestingly, alterations in α_7 nAChRs are believed to contribute to the deficits in sensory gating, sustained attention, and cognitive performance in schizophrenia (reviewed, Freedman 2004). Therefore, it is interesting to hypothesize that a

cognitively impaired psychiatric patient that has been chronically treated with risperidone (where baseline α_7 nAChR activity may be decreased) would be particularly responsive to an α_7 nAChR agonist or an AChEI as an adjunctive agent. Conversely, a patient who has been treated with olanzapine might be less responsive to an AChEI, where olanzapine-related elevations in the M₂ autoreceptor might limit the effectiveness of the increases in synaptic acetylcholine. Obviously, such assertions are speculative at this point, but they represent the types of patient specific (i.e., tailored) therapeutic strategies that should be investigated further.

Antipsychotics Crossover: Therapeutic Implications

When antipsychotic therapy is ineffective, the patient's lack of adherence to the treatment regimen is commonly cited as the basis for the failure. Accordingly, many contemporary clinical studies focus on drug compliance and on methods of ensuring compliance (e.g., depot drug injections). However, the results of our animal studies (where compliance is not a concern) clearly indicate that chronic (uninterrupted) antipsychotic treatment with representative FGAs and SGAs can lead to undesirable effects on neurotrophins, cholinergic proteins, and cognitive function. In addition, we have recently published evidence to indicate that changing from one antipsychotic drug to another during chronic treatment can significantly reduce the extent of NGF and BDNF deficits that occur when a single antipsychotic is given continuously (Pillai et al., 2006). Such results could have important implications for the chronic effects of antipsychotics on CNS cholinergic function as well as cognition. In clinical practice, switching antipsychotic drugs to achieve the best balance between efficacy and side effects is a common occurrence. Other reasons for switching antipsychotics include (interestingly) poor patient compliance, cost (especially with SGAs), and unresponsiveness of cognitive symptoms. It should be noted, that while the practices described above are common, few randomized and

controlled clinical trials have been published to guide the clinician as to the best protocols to follow (reviewed, Remington et al., 2005). Future animal experiments are planned in our laboratories to specifically address this issue by assessing the cellular and biochemical consequences (and effects on cognitive function) of switching between SGAs. The results of such studies would be expected to provide initial (preclinical) indicators as to which compounds would be better choices should a drug switch be indicated.

Conclusions

Schizophrenia is a devastating mental illness that is associated with a lifetime of disability and for sufferers of this illness to function in society, the amelioration of psychotic symptoms is imperative. Fortunately, a variety of antipsychotic drugs have been shown to be effective in this regard; however, multi-year, prospective clinical studies designed specifically to identify the antipsychotics that have optimal effects on cognition have not been conducted (and generally would be cost prohibitive). Therefore, relevant animal studies such as those described in this review are essential since they allow for meticulous time course experiments in antipsychotic naïve subjects (a rarity in schizophrenia patients), the rigorous control of environmental conditions (eliminating confounding clinical factors such as substance abuse, poor nutrition, etc.) and the evaluation of antipsychotic effects on neurobiological substrates of cognition (i.e., at the cellular and molecular level). A growing body of evidence from such animal studies indicates that several antipsychotics including both FGAs and FGAs (if administered for sufficient periods of time) can be associated with impairments in memory-related task performance as well as alterations in central cholinergic function. Given the well-documented importance of central cholinergic neurons to information processing and cognitive function, it is important that the mechanisms of such chronic antipsychotic effects be identified. A better understanding of these

mechanisms would be expected to facilitate optimal treatment strategies to maintain or improve cognitive function in schizophrenia as well as to provide a more educated approach to psychiatric drug discovery and development.

References

- Addy N, Levin ED. (2002) Nicotine interactions with haloperidol, clozapine and risperidone and working memory function in rats. *Neuropsychopharmacology* **27**:534-541.
- Alcantara AA, Chen V, Herring BE, Mendenhall JM, Berlanga ML. (2003) Localization of dopamine D2 receptors on cholinergic interneurons of the dorsal striatum and nucleus accumbens of the rat. *Brain Res* **986**:22-229.
- Angelucci F, Luigi A, Gruber SHM, Fiore M, Mathe AA. (2000) Chronic antipsychotic treatment selectively alters nerve growth factor and neuropeptide Y immunoreactivity and the distribution of choline acetyl transferase in rat brain regions. *Int J Neuropsychopharmacology* **3**:13-25.
- Angelucci F, Brene S, Mathe AA. (2005) BDNF in schizophrenia, depression and corresponding animal models. *Mol Psychiatry* **10**:345-352.
- Beatty WW, Rush JR. (1983) Spatial working memory in rats: effects of monoaminergic antagonists. *Pharmacol Biochem Behav* **18**:7-12.
- Bymaster FP, Felder CC, Tzavara E, Nomikos GG, Calligaro DO, Mckinzie DL. (2003) Muscarinic mechanisms of antipsychotic atypicality. *Prog Neuropsychopharmacol Biol Psychiatry* **27**:1125-1143.
- Carpenter WT, Gold JM. (2002) Another view of therapy for cognition in schizophrenia. *Biol Psychiatry* **52**:969-971.
- Counts SE, Mufson EJ. (2005) The role of nerve growth factor receptors in cholinergic basal forebrain degeneration in prodromal Alzheimer disease. *J Neuropathol Exp Neurol* **64**:263-272.

- Day JC, Fibiger HC. (1994) Dopaminergic regulation of septohippocampal cholinergic neurons. *J Neurochem* **63**:2086-2092.
- Didriksen M. (1995) Effects of antipsychotics on cognitive behaviour in rats using the delayed non-match to position paradigm. *Eur J Pharmacol* **281**:241-250.
- Didriksen M, Kreilgaard M, Arnt J (2006) Sertindole, in contrast to clozapine and olanzapine, does not disrupt water maze performance after acute or chronic treatment. *Eur J Pharmacol* **542**:108-115.
- Fahnestock M, Garzon D, Holsinger RM, Michalski B. (2002) Neurotrophic factors and Alzheimer's disease: are we focusing on the wrong molecule? *J Neural Transm Suppl* **62**:241-252.
- Friedman JI, Adler DN, Howanitz E, Harvey PD, Brenner G, Temporini H, White L, Parrella M, Davis KL. (2002) A double blind placebo controlled trial of donepezil adjunctive treatment to risperidone for the cognitive impairment of schizophrenia. *Biol Psychiatry* **51**:349-357.
- Friedman JI. (2004) Cholinergic targets for cognitive enhancement in schizophrenia: focus on cholinesterase inhibitors and muscarinic agonists. *Psychopharmacology (Berl)* **174**:45-53.
- Gardner DM, Baldessarini RJ, Waraich P. (2005) Modern antipsychotic drugs: a critical overview. *CMAJ*: **172**:1703-1711.
- Gold PE. (2003) Acetylcholine modulation of neural systems involved in learning and memory. *Neurobiology of Learning and Memory* **80**:194-210.
- Green MF, Kern RS, Braff DL, Mintz J. (2000) Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the "right stuff"? *Schizophr Bull* **26**:119-136.

- Harvey PD, Green MF, Keefe RS, Velligan DI. (2004) Cognitive functioning in schizophrenia: a consensus statement on its role in the definition and evaluation of effective treatments for the illness. *J Clin Psychiatry* **65**:361-672.
- Ichikawa J, Dai J, O'Laughlin IA, Fowler WL, Meltzer HY (2002). Atypical, but not typical, antipsychotic drugs increase cortical acetylcholine release without an effect in the nucleus accumbens or striatum. *Neuropsychopharmacology* **26**:325-339.
- Keefe RS, Young CA, Rock SL, Purdon SE, Gold JM, Breier A; HGGN Study Group (2006). One-year double-blind study of the neurocognitive efficacy of olanzapine, risperidone, and haloperidol in schizophrenia. *Schizophr Res* **81**:1-15.
- Kitabatake Y, Hikida T, Watanabe D, Pastan I, Nakanishi S. (2003) Impairment of reward-related learning by cholinergic cell ablation in the striatum. *Proc Natl Acad Sci* **100**:7965-7970.
- Levin ED, Galen DM, Ellison GD. (1987) Chronic haloperidol effects on oral movements and radial-arm maze performance in rats. *Pharmacol Biochem Behav* **26**:1-6.
- Levin ED, Petro A, Beatty A. (2005) Olanzapine interactions with nicotine and mecamylamine in rats: effects on memory function. *Neurotoxicol Teratol* **27**:459-464.
- Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Keefe RS, Davis SM, Davis CE, Lebowitz BD, Severe J, Hsiao JK. (2005) Clinical antipsychotic trials of intervention effectiveness (CATIE) investigators effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N Engl J Med*. **353**:1209-1223.
- Lipska BK, Khaing ZZ, Weickert CS, Weinberger DR. (2001) BDNF mRNA expression in rat hippocampus and prefrontal cortex: effects of neonatal ventral hippocampal damage and antipsychotic drugs. *Eur J Neurosci* **14**:135-144.

- Mahadik SP, Laev H, Korenovsky A, Karpiak SE. (1988) Haloperidol alters rat CNS cholinergic system: enzymatic and morphological analyses. *Biol Psychiatry* **24**:199-217.
- Miller R, Chouinard G. (1993) Loss of striatal cholinergic neurons as a basis for tardive and L-dopa-induced dyskinesias, neuroleptic-induced supersensitivity psychosis and refractory schizophrenia. *Biol Psychiatry* **34**:713-738.
- Miyamoto S, Duncan GE, Marx CE, Lieberman JA. (2005) Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol Psychiatry* **10**:79-104.
- Nuechterlein KH, Dawson ME, Gitlin M, Ventura J, Goldstein MJ, Snyder KS, Yee CM, Mintz J. (1992) Developmental processes in schizophrenic disorders: longitudinal studies of vulnerability and stress. *Schizophr Bull* **18**:387-425.
- Paxinos G, Watson C (2005) *The rat brain in stereotaxic coordinates*, 5th ed. Elsevier Academic Press, San Diego, CA:
- Pillai A, Terry AV Jr, Mahadik SP. (2006) Differential effects of long-term treatment with typical and atypical antipsychotics on NGF and BDNF levels in rat striatum and hippocampus. *Schizophr Res* **82**:95-106.
- Ploeger GE, Spruijt BM, Cools AR. (1992) Effects of haloperidol on the acquisition of a spatial learning task. *Physiol Behav* **52**:979-983.
- Remington G, Chue P, Stip E, Kopala L, Girard T, Christensen B. (2005) The crossover approach to switching antipsychotics: what is the evidence? *Schizophr Res* **76**:267-272.
- Rosengarten H, Quartermain D. (2002) The effect of chronic treatment with typical and atypical antipsychotics on working memory and jaw movements in three- and eighteen-month-old rats. *Prog Neuropsychopharmacol Biol Psychiatry* **26**:1047-1054.

- Sarter M. and Bruno JP. (1999) Abnormal regulation of corticopetal cholinergic neurons and impaired information processing in neuropsychiatric disorders. *Trends Neurosci* **22**: 67-74.
- Sarter M, Nelson CL, Bruno JP. (2005) Cortical cholinergic transmission and cortical information processing in schizophrenia. *Schizophr Bull* **31**:117-138.
- Schubert MH, Young KA, Hicks PB. (2006) Galantamine improves cognition in schizophrenic patients stabilized on risperidone. *Biol Psychiatry* Jun 23; [Epub ahead of print] doi:10.1016/j.biopsych.2006.04.006.
- Sharma T, Mockler D. (1998) The cognitive efficacy of atypical antipsychotics in schizophrenia. *J Clin Psychopharmacol* **18** (2 Suppl 1):12S-9S.
- Sharma T, Reed C, Aasen I, Kumari V. (2006) Cognitive effects of adjunctive 24-weeks rivastigmine treatment to antipsychotics in schizophrenia: A randomized, placebo-controlled, double-blind investigation. *Schizophr Res* **85**:73-83.
- Shirazi-Southall S, Rodriguez DE, Nomikos GG. (2002) Effects of typical and atypical antipsychotics and receptor selective compounds on acetylcholine efflux in the hippocampus of the rat. *Neuropsychopharmacology* **26**:583-594.
- Skarsfeldt T. (1996) Differential effect of antipsychotics on place navigation of rats in the Morris water maze. A comparative study between novel and reference antipsychotics. *Psychopharmacology* **124**:126-133.
- Squire, LR (1994) Declarative and non-declarative memory: multiple brain systems supporting learning and memory, in Schacter, D.L., Tulving, E. (Eds.), *Memory Systems* MIT Press, Cambridge, MA, pp. 203–231.
- Suzuki T, Miura M, Nishimura K, Aosaki T. (2001) Dopamine-dependent synaptic plasticity in the striatal cholinergic interneurons. *J Neurosci* **21**:6492-6501.

- Terry AV, Jr ., Gearhart DA, Mahadik SP, Warsi S, Davis, LW, Waller, JL. (2005a) Chronic exposure to typical or atypical antipsychotics in rodents: temporal effects on central alpha 7 nicotinic acetylcholine receptors. *Neuroscience* **136**:519-529.
- Terry AV Jr, Gearhart DA, Mahadik SP, Warsi S, Waller JL. (2006a) Chronic treatment with first or second generation antipsychotics in rodents: effects on high affinity nicotinic and muscarinic acetylcholine receptors in the brain. *Neuroscience* **140**:1277- 1287.
- Terry Jr AV, Parikh V, Gearhart DA, Pillai A, Hohanadel E, Warner S, Nasrallah HA, Mahadik SP. (2006b) Time dependent effects of haloperidol and ziprasidone on nerve growth factor, cholinergic neurons, and spatial learning in rats. *J Pharmacol Exp Ther* **318**:709-724.
- Wilk CM, Gold JM, Humber K, Dickerson F, Fenton WS, Buchanan RW. (2004) Brief cognitive assessment in schizophrenia: normative data for the repeatable battery for the assessment of neuropsychological status. *Schizophr Res* **70**:175-186.
- Wolff MC, Leander JD. (2003) Comparison of the effects of antipsychotics on a delayed radial maze task in the rat. *Psychopharmacology (Berl)* **168**:410-416.
- Youngblood BD, Zhou J, Smagin GN, Ryan DH, Harris RBS. (1997) Sleep deprivation by the “flower pot” technique and spatial reference memory. *Physiol Behav* **61**:249-256.

Footnotes:

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Legends for Figures

Fig 1. First and second generation antipsychotics are associated with time-dependent impairments of water maze task acquisition in rats. Rats were treated with antipsychotic drugs chronically in their drinking water, given a one week washout period, and tested in a water maze task daily for an additional week. Top Figures = haloperidol (HAL, 2.0 mg/kg/day) and risperidone (RISP, 2.5 mg/kg/day) comparisons to vehicle controls; Bottom Figures = chlorpromazine (CPZ, 10.0 mg/kg/day) and olanzapine (OLZ, 10.0 mg/kg/day) comparisons to vehicle controls. The latency AUC for water maze hidden platform test results across 3 time periods of drug treatment were calculated and statistically compared. Each bar represents the mean \pm SEM. There was a highly significant effect of treatment ($p < 0.001$), time of drug administration ($p < 0.001$), and the time \times treatment interaction was significant ($p < 0.02$) in both studies. * = significantly different ($p < 0.05$) from vehicle control value within a session. + = significantly different from the 45 day time point (within treatment). The statistical test used was a two-way ANOVA with Bonferroni's Test for post hoc comparisons. N=8 for all groups at the 45 day time point and N=12 for all other groups.

Fig. 2. First and second generation antipsychotics are associated with persistent decreases in cholinergic marker proteins in the rat brain. Rats were treated orally with antipsychotics for 90 days, given a given a two week washout period, and sacrificed for measurements of cholinergic marker proteins in the brain. A = ChAT immunostaining of 40 μ m coronal sections of cortex (CTX) or caudate-putamen (CP). Diagram (Bregma 1.68 mm) is adapted from Paxinos and Watson rat brain atlas, 2005). The bar represents approximately 5 μ m in the cortex and 10 μ m in CP. B = Results of ELISA measurements of the vesicular acetylcholine transporter (VACHT) in

homogenates of basal forebrain and prefrontal cortex. Each bar (expressed as a % of vehicle control) represents the mean \pm SEM for each test group. *=significantly different from vehicle control, $p < 0.05$. The statistical test used was a one-way ANOVA. V or VEH = vehicle controls; H or HAL=haloperidol; R or RISP = risperidone; ZIP=ziprasidone; CPZ=chlorpromazine; OLZ=olanzapine. N=5-6 for all groups.

Fig. 3. First and second generation antipsychotics are associated with time-dependent and persistent decreases in NGF levels in the hippocampus. Rats were treated with antipsychotic drugs chronically in their drinking water, given a two week washout period, and sacrificed for NGF measurements. A = NGF immunostaining of 20 μm coronal sections after 90 days of treatment. The bar = approximately 80 μm for the DG (dentate gyrus) and 100 μm for the CA1 and CA3 regions. B = Results of ELISA experiments on whole hippocampal homogenates after 45, 90, or 180 days of antipsychotic treatment. Each bar represents the mean \pm SEM for each test group. *=significantly different from vehicle control $p < 0.05$; **= $p < 0.01$. The statistical test used was a one-way ANOVA. V or VEH = vehicle controls; H or HAL=haloperidol; R or RISP = risperidone; Z=ziprasidone; CPZ=chlorpromazine; OLZ=olanzapine. N=5-6 for all groups

Fig 1

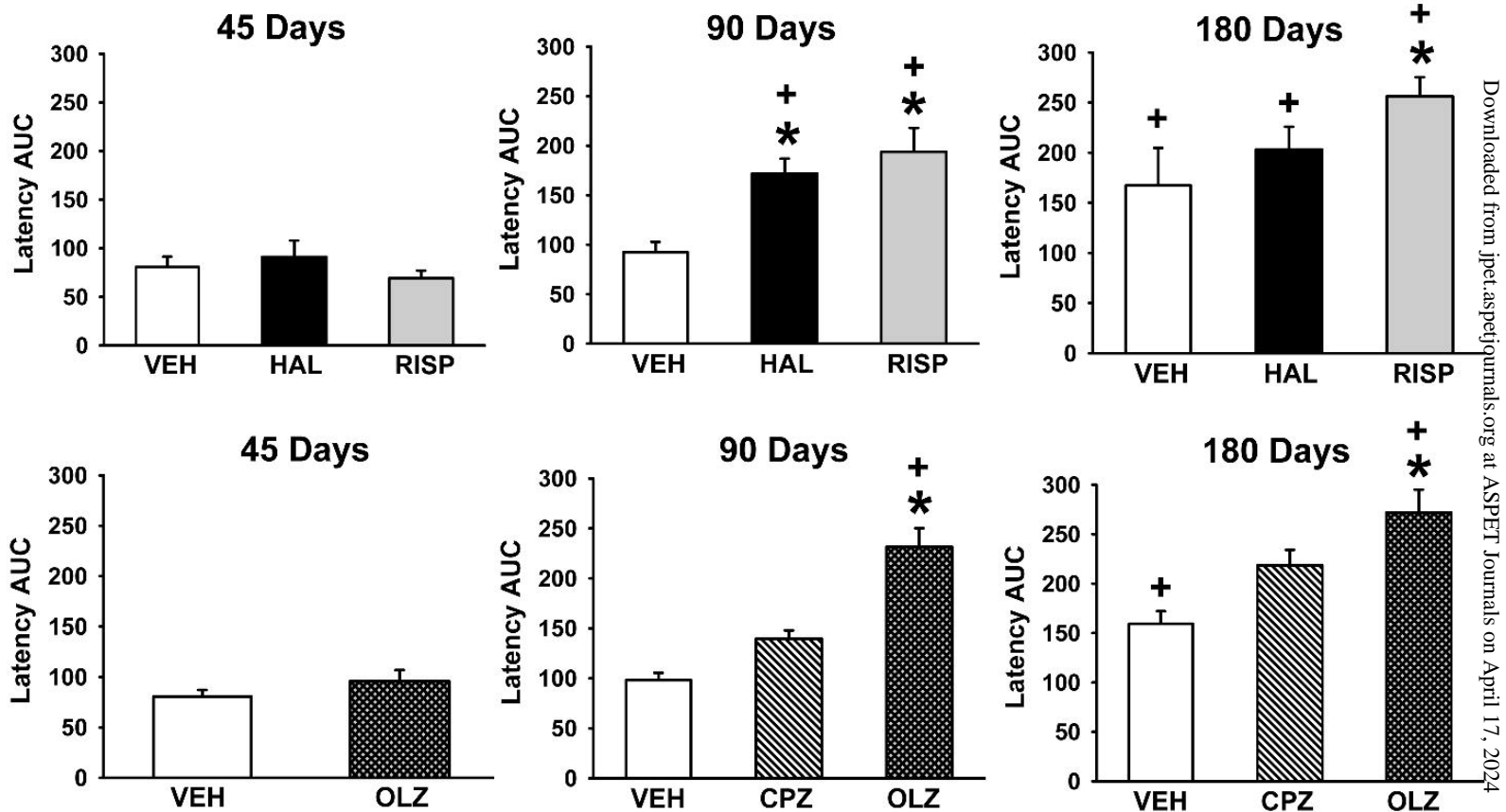


Fig 2

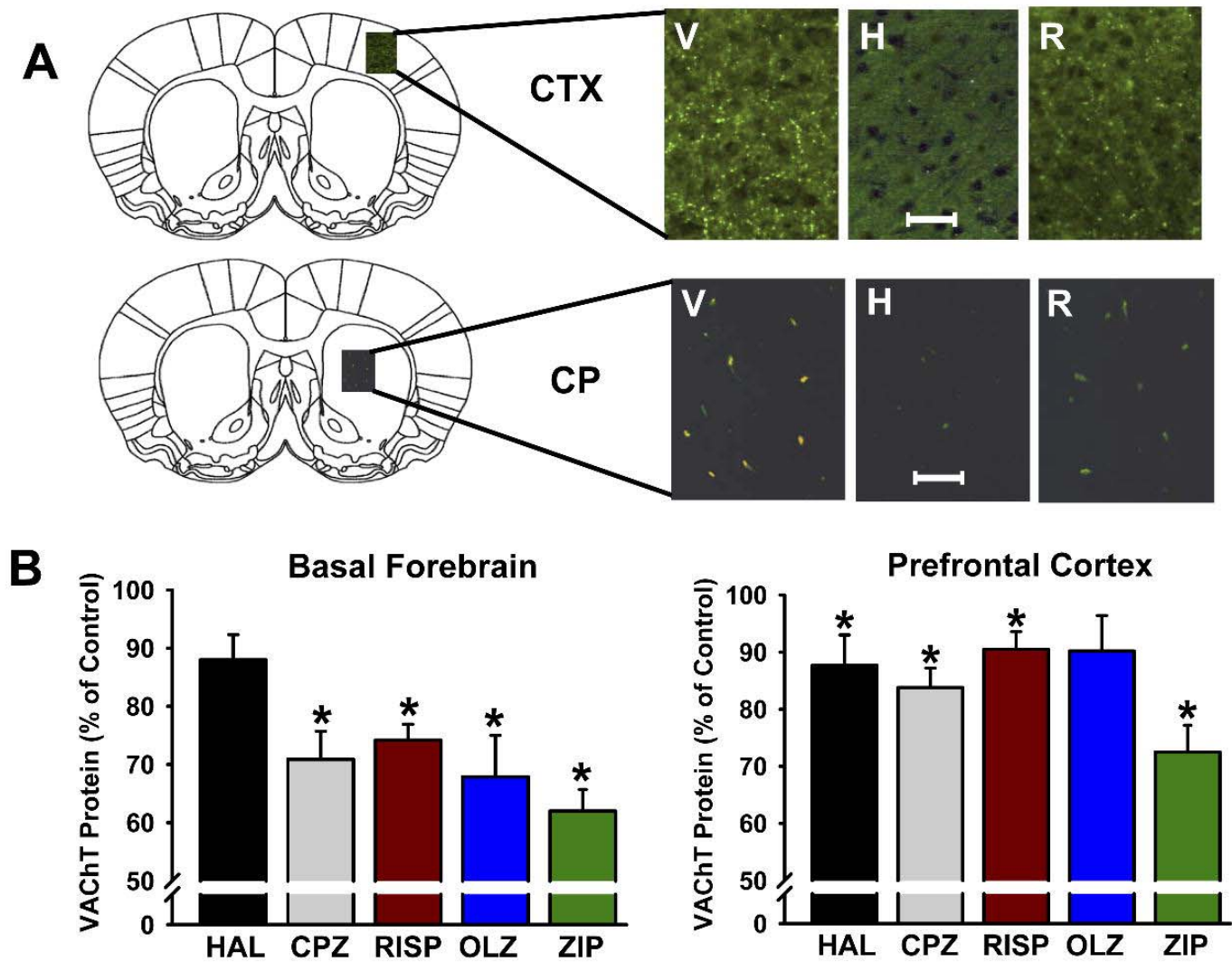


Fig 3

