THE CENTRAL CHOLINERGIC SYSTEM AS A THERAPEUTIC TARGET FOR THE COGNITIVE DYSFUNCTION OF SCHIZOPHRENIA

by

ELIZABETH JOAN (HOHNADEL) HERMAN

(Under the Direction of Alvin V. Terry, Jr., Ph.D.)

Schizophrenia is characterized by three core symptoms: positive, negative and cognitive dysfunction, the latter symptom is best correlated with long term functional outcome. While most studies related to schizophrenia (including those related to cognitive function) have focused on abnormalities in the neurotransmitters, dopamine, serotonin and glutamate, there is also evidence that acetylcholine neurotransmission (i.e., central cholinergic function) is altered in the illness. Thus, the central cholinergic system could serve as a therapeutic target for improving cognition in schizophrenia. In the evaluation of two commonly used AChEIs for effects on sensorimotor gating in an experimental animal model, galantamine (depending on dose) improved PPI deficits in three pharmacologic models of PPI impairment, whereas donepezil ameliorated PPI deficits induced by scopolamine and apomorphine, but was not effective in the MK801 model. In radial arm maze experiments neither haloperidol nor risperidone affected win-shift acquisition although DNMTP performance was modestly impaired at the longer delays by risperidone. Haloperidol, but not risperidone, impaired water maze hidden platform acquisition as well as probe trial performance possibly due to psychomotor impairments and elevated levels of anxiety. In 5CSRTT experiments haloperidol and, to a lesser degree, risperidone, impaired task acquisition as indicated by the failure (or increase in the number of trials) to meet specific performance criteria. Results from

these behavior experiments indicate task dependent and temporal effects of exposure to therapeutic doses of haloperidol and risperidone and risperidone may impair spatial working and short-term memory as the demands of the task increase. Haloperidol and risperidone were without significant effect in the ELISA experiments which were conducted to detect potential (antipsychotic-related) alterations in the levels of VAChT and α_7 nAChR in medial prefrontal cortex of rats treated for 320 days.

INDEX WORDS: Schizophrenia, Acetylcholine, Antipsychotics, Cholinesterase Inhibitors, Prepulse inhibition, Radial arm maze, Morris water maze, 5-Choice Serial Reaction Time Task, Wistar rats, VAChT

THE CENTRAL CHOLINERGIC SYSTEM AS A THERAPEUTIC TARGET FOR THE COGNITIVE DYSFUNCTION OF SCHIZOPHRENIA

by

ELIZABETH JOAN (HOHNADEL) HERMAN

B.S., Presbyterian College, 1992

M.Ed., Georgia College & State University, 1998

M.S., Georgia College & State University 2002

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2007

THE CENTRAL CHOLINERGIC SYSTEM AS A THERAPEUTIC TARGET FOR THE COGNITIVE DYSFUNCTION OF SCHIZOPHRENIA

by

ELIZABETH JOAN (HOHNADEL) HERMAN

Major Professor: Alvin V. Terry, Jr

Committee: Susan C. Fagan
Adviye Ergul
Randall L. Tackett
Cesario Borlongan

Electronic Version Approved:

Maureen Grasso

Dean of the Graduate School

The University of Georgia

December 2007

DEDICATION

This dissertation is dedicated to my loving husband, Chad, and my beautiful daughter, Grace, and my amazing parents, E.J. & Carolyn Harrelson.

ACKNOWLEDGEMENTS

American Foundation for Pharmaceutical Education

National Institute of Mental Health (MH 066233 to AVT)

Dr. Alvin V. Terry, Jr.

Technicians of Dr. Terry's lab

Technicians of MCG Small Animal Behavior Core

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Schizophrenia affects 1% of the world's population and ranks among the top ten causes of disability in developed countries. Due to its chronic and disabling nature, the disease accounts for one fourth of all mental health costs in the United States (estimated by the National Institutes of Mental Health at $65 billion for the 2.2 million sufferers). While schizophrenia is characterized by three core symptoms: positive, negative and cognitive dysfunction, the latter symptom is best correlated with long term functional outcome. Unfortunately, while many of the available pharmacological treatments are effective at decreasing psychotic symptoms of schizophrenia, none have been conclusively demonstrated to improve the cognitive dysfunction or the associated functional impairments. While most studies related to schizophrenia (including those related to cognitive function) have focused on abnormalities in the neurotransmitters, dopamine, serotonin and glutamate, there is also evidence that acetylcholine neurotransmission (i.e., central cholinergic function) is altered in the illness. Thus, the central cholinergic system could serve as a therapeutic target for improving cognition in schizophrenia.

LITERATURE REVIEW

I. Cognitive Deficits of Schizophrenia

Cognitive deficits have moved to the forefront of drug targeting in schizophrenia. They are now recognized as a third group of core symptoms accompanying positive and negative signs that characterize this disease. Cognitive deficits actually dictate the severity of the disorder itself as well as predict the functional outcome of schizophrenics (reviewed Green, 1996; Kurtz et al., 2005; Pinkham and Penn, 2006; Villalta-Gil et al.,

2006). Concern regarding cognitive deficits prompted the National Institute of Mental Health (NIMH) to organize a team of researchers to discern the areas of cognitive deficits found in schizophrenia and to dictate appropriate standard testing procedures for each factor. In MATRICS (Measurement And Treatment Research to Improve Cognition in Schizophrenia), this team recognized eight separable cognitive factors: Speed of Processing, Attention/Vigilance, Working Memory, Verbal Learning and Memory, Visual Learning and Memory, Reasoning and Problem Solving, Verbal Comprehension, and Social Cognition (Nuechterlein et al., 2004). In my work outlined in this proposal, I have concentrated on how speed of processing, attention and vigilance, and working memory are altered through treatment with haloperidol and risperidone, two commonly prescribed antipsychotics. Spatial learning, also disrupted in schizophrenia, is yet another component of some tests utilized in this project to evaluate effects of antipsychotic treatment on cognition. Successful amelioration of cognitive deficits could significantly improve the quality of life of patients by enabling them to live more productively and independently. This would in turn lessen the emotional and financial burden this disease places on family members and society as a whole.

II. Neuroleptic Therapy

Current therapies for schizophrenia effectively control positive symptoms well and negative symptoms to a moderate degree. First generation antipsychotics (FGAs), also termed typical, developed to treat schizophrenia were predominantly dopamine receptor antagonists. These include perphenazine and haloperidol. Due to the high incidence of extrapyramidal symptoms (EPS), FGAs have mostly been replaced by atypical or second generation antipsychotics (SGAs), drugs that began entering the market in the

1980's. Examples of these are clozapine, quetiapine, olanzapine, risperidone, ziprasidone, and aripiprazole. These agents are antagonists at dopamine receptors (D_2) but also act as antagonists at serotonin receptors ($5HT_{2A}$). This additional mechanism has been hypothesized to decrease EPS (Kessler et al., 2005, reviewed by Seeman, 2002) and potentially result in a more favorable effect on cognition. However, weight gain, hyperlipidemia, and diabetes mellitus (reviewed Shirzadi and Ghaemi, 2006) have emerged as significant side effects associated with SGAs.

The discovery that SGAs have many adverse effects, some of them potentially life threatening with time, has researchers and clinicians reconsidering the choice of SGAs as first-line therapy over FGAs. This debate has been further fueled by the focus on cognitive improvement, methodological weaknesses from earlier studies with haloperidol, and the expense of the newer SGAs. More recent studies have established that lower doses of haloperidol may have positive effects on neurocognition (Green et al., 2002; Keefe et al., 2004) similar to risperidone (Green et al., 2002) in chronic schizophrenia patients but not as significant as olanzapine (Keefe et al., 2004) or risperidone (Harvey et al., 2003) in first episode patients. As measured by improvements in mental status to a point where patients no longer required hospitalization and by the Brief Psychiatric Rating Scale scores, McCue et al. (2006) found haloperidol, olanzapine, and risperidone superior to aripiprazole**,** quetiapine, and ziprasidone for acute treatment of psychosis in hospitalized patients with schizophrenia, schizoaffective disorder or schizophreniform disorder. In a one year study comparing treatment with haloperidol, risperidone, or olanzapine, all patient groups displayed

neurocognitive improvement, but olanzapine and risperidone treatment positively influenced more domains than haloperidol (Keefe et al., 2006). It is yet unclear what therapy offers the highest efficacy and least adverse effects.

III. Cholinergic System and Schizophrenia

Treatment for schizophrenia is complicated by the complexity of the disorder itself. Pathologic hypotheses exist citing dysfunction of the glutamate (see reviews by Kondziella et al., 2007; Laruelle et al., 2003; and Lindsley et al., 2006), GABA (review by Caruncho et al., 2004), dopamine (reviews by Carlsson and Carlsson, 2006; Horacek et al., 2006) and/or serotonin (Akhondzadeh, 2001) neurotransmitter systems as underlying the etiology of the disease. Interestingly, there is some evidence to suggest that cholinergic function is also altered in schizophrenia. For example, significant correlations between reduced choline acetyltransferase (i.e., ChAT, the acetylcholine synthesizing enzyme) levels and impaired cognition in schizophrenia have been observed (reviewed, Powchik et al., 1998). Moreover, decreases in both low affinity (α_7) nicotinic acetylcholine receptors (nAChRs) in the hippocampus and high affinity ($\alpha_4\beta_2$) nAChRs in the hippocampus, cortex, striatum, and thalamus have been detected in postmortem brains of schizophrenics (reviewed by Freedman et al., 2000; Ripoll et al., 2004). The number of M_1/M_4 muscarinic acetylcholine receptors (mAChRs) has also been found to be reduced relative to normal postmortem brains in several areas including the hippocampus (Crook et al., 2000), prefrontal cortex (Crook et al., 2001) and striatum (Dean et al., 1996). Likewise, single photon emission computed tomography (SPECT) in living, unmedicated schizophrenic patients revealed fewer mAChRs in frontal, temporal, and occipital cortex as well as in the striatum and

thalamus compared to control subjects (Raedler et al., 2003). In fact, many investigational therapies targeting cognitive improvement have been cholinergic substances, muscarinic and nicotinic receptor agonists along with acetylcholinesterase inhibitors.

The cholinergic alterations described above may have particular importance to certain features of schizophrenia such as sensory gating abnormalities believed to contribute to deficits in attention, cognitive impairment, and possibly hallucinations (Adler et al., 1998). Behavioral tests for this project were chosen based on their known sensitivity to alterations in cholinergic function and their ability to assess specific domains of cognition that are known to be affected in schizophrenia. The disruption of “prepulse inhibition” (PPI), a well characterized model for identifying sensory information-processing deficits, is in fact, clearly established in schizophrenia (reviewed, Braff et al., 2001). The fact that PPI is a cross-species phenomenon also allows for translation between animal experiments and clinical studies (Swerdlow et al., 2001). Deficits in focused attention, vigilance, and divided attention can be assessed in rodents using the 5-Choice Serial Reaction Time Task (5CSRTT) analogous to the Continuous Performance Task used for humans (for review see Robbins, 2002). Inadequate inhibitory response control (an important component of executive function) as quantified through premature and perseverative responses can also be measured through 5CSRTT. Working memory can be evaluated in rodents through the radial arm maze and spatial learning can be assessed through the Morris water maze. Many verbal and nonverbal human counterparts to these methods exist, and virtual mazes similar to those used in animal labs have been developed and tested in humans.

In this project, I investigate three essential aspects of schizophrenia treatment. First, behavioral testing in the proposal applies methods examining three progressive (and interdependent) phases of cognition, each appearing to be deficient in schizophrenia; these are pre-attentive processing, attention, and working memory. Second, I assess the effects of chronic antipsychotic treatment with one FGA and one SGA on acquisition as well as performance of tasks. This provides clinical relevance since patient employment opportunities (and other important activities of daily life) constantly require the mastering of new skills. Third, the length of drug exposure, spanning 320 days, has seldom, if ever, been conducted in a rodent model. Relative to the life span of a human, this is the equivalent of beginning treatment in late adolescence/early adulthood and continuing to middle age. This is extremely pertinent due to initiation treatment in clinical populations close to disease onset (typically late adolescence/early adulthood) and its continuation throughout their lives. With trends advocating treatment earlier and earlier for schizophrenia to prevent further damage to cognition as well as more frequent relapses (Malla et al., 2002; Clarke et al., 2006; review by Marshall and Rathbone, 2006), antipsychotics are prescribed to increasingly younger populations, extending treatment periods.

References

Adler LE, Olincy A, Waldo M, Harris JG, Griffith J, Stevens K, Flach K, Nagamoto H, Bickford P, Leonard S and Freedman R (1998) Schizophrenia, sensory gating, and nicotinic receptors. *Schizophr Bull* **24**:189-202.

Akhondzadeh S (2001) The 5-HT hypothesis of schizophrenia. *Drugs* **4**:295-300.

Braff DL, Geyer MA and Swerdlow NR (2001) Human studies of prepulse inhibition of startle: Normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)* **156**:234-258.

Carlsson A and Carlsson ML (2006) A dopaminergic deficit hypothesis of schizophrenia: The path to discovery. *Dialogues Clin Neurosci* **8**:137-142.

Caruncho HJ, Dopeso-Reyes IG, Loza MI and Rodriguez MA (2004) A GABA, reelin, and the neurodevelopmental hypothesis of schizophrenia. *Crit Rev Neurobiol* **16**:25-32.

Clarke M, Whitty P, Browne S, McTigue O, Kamali M, Gervin M, Kinsella A, Waddington JL, Larkin C and O'Callaghan E (2006) Untreated illness and outcome of psychosis. *Br J Psychiatry* **189**:235-240.

Crook JM, Tomaskovic-Crook E, Copolov DL and Dean B (2000) Decreased muscarinic receptor binding in subjects with schizophrenia: A study of the human hippocampal formation. *Biol Psychiatry* **48**:381-388.

Crook JM, Tomaskovic-Crook E, Copolov DL and Dean B (2001) Low muscarinic receptor binding in prefrontal cortex from subjects with schizophrenia: A study of Brodmann's areas 8, 9, 10, and 46 and the effects of neuroleptic drug treatment. *Am J Psychiatry* **158**:918-925.

Dean B, Crook JM, Opeskin K, Hill C, Keks N and Copolov DL (1996) The density of muscarinic M1 receptors is decreased in the caudate-putamen of subjects with schizophrenia. *Mol Psychiatry* **1**:54-58.

Freedman R, Adams CE and Leonard S (2000) The alpha7-nicotinic acetylcholine receptor and the pathology of hippocampal interneurons in schizophrenia. *J Chem Neuroanat* **20**:299-306.

Gearhart DA, Middlemore ML and Terry AV (2006) ELISA methods to measure cholinergic markers and nerve growth factor receptors in cortex, hippocampus, prefrontal cortex, and basal forebrain from rat brain. *J Neurosci Methods* **150**:159-173.

Geerts H, Guillaumat P-O, Grantham C, Bode W, Anciaux K and Sachak S (2005) Brain levels and acetylcholinesterase inhibition with galantamine and donepezil in rats, mice, and rabbits. *Brain Research* **1033**:186-193.

Green M, Marder S, Glynn S, McGurk S, Wirshing W, Wirshing D, Liberman R and Mintz J (2002) The neurocognitive effects of low-dose haloperidol: A two-year comparison with risperidone. *Biol Psychiatry* **51**:972-978.

Green MF (1996) What are the functional consequences of neurocognitive deficits in schizophrenia? *Am J Psychiatry* **153**:321-330.

Harvey PD, Green MF, McGurk SR and Meltzer HY (2003) Changes in cognitive functioning with risperidone and olanzapine treatment: A large-scale, double-blind, randomized study. *Psychopharmacol (Berl)* **169**:404-411.

Horacek J, Bubenikova-Valesova V, Kopecek M, Palenicek T, Dockery C, Mohr P and

Hoschl C (2006) Mechanism of action of atypical antipsychotic drugs and the neurobiology of schizophrenia. *CNS Drugs* **20**:389-409.
Kapur S, VanderSpek SC, Brownlee BA and Nobrega JN (2003) Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: A suggested solution based on in vivo occupancy. *J Pharmacol Exp Ther* **305**:625-631.
Keefe RS, Seidman LJ, Christensen BK, Hamer RM, Sharma T, Sitskoorn MM, Lewine RR, Yurgelun-Todd DA, Gur RC, Tohen M, Tollefson GD, Sanger TM and Lieberman JA (2004) Comparative effect of atypical and conventional antipsychotic drugs on neurocognition in first-episode psychosis: a randomized, double-blind trial of olanzapine versus low doses of haloperidol. *Am J Psychiatry* **161**:985-995.
Keefe RS, Young CA, Rock SL, Purdon SE, Gold JM and Breier A (2006) One-year double-blind study of the neurocognitive efficacy of olanzapine, risperidone, and haloperidol in schizophrenia. *Schizophr Res* **81**:1-15.
Kessler RM, Ansari MS, Riccardi P, Li R, Jayathilake K, Dawant B and Meltzer HY (2005) Occupancy of striatal and extrastriatal dopamine D2//D3 Receptors by olanzapine and haloperidol. *Neuropsychopharmacol* **30**:2283-2289.
Kondziella D, Brenner E, Eyjolfsson E and Sonnewald U (2007) How do glial-neuronal interactions fit into current neurotransmitter hypotheses of schizophrenia? *Neurochem Int* **50**: 291-301.
Kurtz MM, Moberg PJ, Ragland JD, Gur RC and Gur RE (2005) Symptoms versus neurocognitive test performance as predictors of psychosocial status in schizophrenia: a 1- and 4-year prospective study. *Schizophr Bull* **31**:167-174.
Laruelle M, Kegeles LS and Abi-Dargham A (2003) Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. *Ann N Y Acad Sci* **1003**:138-158.
Lindsley CW, Shipe WD, Wolkenberg SE, Theberge CR, Williams DL, Jr., Sur C and Kinney GG (2006) Progress towards validating the NMDA receptor hypofunction hypothesis of schizophrenia. *Curr Top Med Chem* **6**:771-785.
Malla AK, Norman RM, Manchanda R, Ahmed MR, Scholten D, Harricharan R, Cortese L and Takhar J (2002) One year outcome in first episode psychosis: influence of DUP and other predictors. *Schizophr Res* **54**:231-242.
Marshall M and Rathbone J (2006) Early intervention for psychosis. *Cochrane Database Syst Rev*: Oct 18;(4):CD004718.
McCue RE, Waheed R, Urcuyo L, Orendain G, Joseph MD, Charles R and Hasan SM (2006) Comparative effectiveness of second-generation antipsychotics and haloperidol in acute schizophrenia. *Br J Psychiatry* **189**:433-440.
Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF and Heaton RK (2004) Identification of separable cognitive factors in schizophrenia. *Schizophr Res* **72**:29-39.
Pinkham AE and Penn DL (2006) Neurocognitive and social cognitive predictors of interpersonal skill in schizophrenia. *Psychiatry Res* **143**:167-178.
Powchik P, Davidson M, Haroutunian V, Gabriel SM, Purohit DP, Perl DP, Harvey PD and Davis KL (1998) Postmortem studies in schizophrenia. *Schizophr Bull* **24**:325-341.
Raedler TJ, Knable MB, Jones DW, Urbina RA, Egan MF and Weinberger DR (2003)

Central muscarinic acetylcholine receptor availability in patients treated with clozapine. *Neuropsychopharmacol* **28**:1531-1537.
Ripoll N, Bronnec M and Bourin M (2004) Nicotinic receptors and schizophrenia. *Curr Med Res Opin* **20**:1057-1074.
Robbins TW (2002) The 5-choice serial reaction time task: Behavioural pharmacology and functional neurochemistry. *Psychopharmacol (Berl)* **163**:362-380.
Seeman P (2002) Atypical antipsychotics: Mechanism of action. *Can J Psychiatry.* **47**:27-38.
Shirzadi AA and Ghaemi SN (2006) Side effects of atypical antipsychotics: extrapyramidal symptoms and the metabolic syndrome. *Harv Rev Psychiatry* **14**:152-164.
Swerdlow NR, Eastvold A, Uyan KM, Ploum Y and Cadenhead K (2001) Matching strategies for drug studies of prepulse inhibition in humans. *Behav Pharmacol* **12**:45-52.
Terry AV, Jr., Gearhart DA, Mahadik SP, Warsi S, Davis LW and Waller JL (2005a) Chronic exposure to typical or atypical antipsychotics in rodents: Temporal effects on central alpha7 nicotinic acetylcholine receptors. *Neuroscience* **136**:519-529.
Terry AV, Jr., Hernandez CM, Hohnadel EJ, Bouchard KP and Buccafusco JJ (2005b) Cotinine, a neuroactive metabolite of nicotine: Potential for treating disorders of impaired cognition. *CNS Drug Rev* **11**:229-252.
Terry AV, Jr., Hill WD, Parikh V, Evans DR, Waller JL and Mahadik SP (2002) Differential effects of chronic haloperidol and olanzapine exposure on brain cholinergic markers and spatial learning in rats. *Psychopharmacoly (Berl)* **164**:360-368.
Terry AV, Jr., Hill WD, Parikh V, Waller JL, Evans DR and Mahadik SP (2003) Differential effects of haloperidol, risperidone, and clozapine exposure on cholinergic markers and spatial learning performance in rats. *Neuropsychopharmacol* **28**:300-309.
Villalta-Gil V, Vilaplana M, Ochoa S, Haro JM, Dolz M, Usall J and Cervilla J (2006) Neurocognitive performance and negative symptoms: Are they equal in explaining disability in schizophrenia outpatients? *Schizophr Res* **87**:246-253.

DISSERTATION OBJECTIVES

There are two major topics that will be addressed in this proposal, 1). the evaluation in an animal model (for some features of schizophrenia) of drugs that enhance central cholinergic function and cognition in other diseases such as dementia; 2). A comparison of commonly used antipsychotic drugs for their effects on the cholinergic system and cognitive function.

Our long-term goal is to develop effective therapeutic strategies to improve cognition in schizophrenia. The **objectives of this project** are to determine if acetylcholinesterase inhibitors (AChEIs) have therapeutic potential as cognitive enhancing agents in an animal model of schizophrenia and if atypical neuroleptics are superior to typical neuroleptics in animal models of cognitive function due to their superior effects on the central cholinergic system. Two **hypotheses will be tested. The first hypothesis** is that enhancement of the cholinergic system by acetylcholinesterase inhibitors will improve cognitive function in an animal behavior procedure that models auditory gating deficits, one feature of schizophrenia. The **second hypothesis** is that due to less deleterious effects on the cholinergic system in the brain, atypical neuroleptics when administered chronically will be superior to typical neuroleptics in animal models of sustained attention and working memory. The **rationale** for the proposed laboratory research is that identifying methods to optimize cholinergic activity in the brain and performance of memory-related tasks in an animal model (i.e., the rat) will facilitate future (clinical) efforts to identify optimal therapies for cognitively impaired psychiatric patients.

To test these hypotheses, the following specific aims will be addressed:

Specific Aim 1. To evaluate two commonly used AChEIs for effects on sensorimotor gating in an experimental animal model.

Specific Aim 2. To evaluate the effects of representatives of different classes of neuroleptic drugs on cognitive function in experimental animal models.

Specific Aim 3. To determine if a relationship exists between neuroleptic-induced effects on the central cholinergic system and the behavioral changes.

CHAPTER 2

GALANTAMINE AND DONEPEZIL ATTENUATE PHARMACOLOGICALLY INDUCED DEFICITS IN PREPULSE INHIBITION IN RATS

Hohnadel, E., K. Bouchard, and A.V. Terry, Jr. 2007. Neuropharmacology. 52(2):542-51.
Reprinted here with permission of publisher.

ABSTRACT

Acetylcholinesterase inhibitors (AChEIs) are currently being evaluated as adjunctive therapy for the cognitive dysfunction of schizophrenia. This core symptom of schizophrenia has often been attributed to impaired attention and abnormal sensory motor gating, features that are also found in Huntington's Disease, autism, and several other psychiatric and neurological disorders. The ability to improve prepulse inhibition (PPI) of the acoustic startle response may predict the efficacy of compounds as cognitive enhancers. In this study, PPI was disrupted in Wistar rats in three pharmacologic models: dopamine receptor agonism by apomorphine, NMDA receptor antagonism by MK-801, or muscarinic acetylcholine receptor antagonism by scopolamine. We then evaluated the commonly used AChEIs, donepezil (0.5, 1.0, or 2.0 mg/kg) and galantamine (0.3, 1.0, or 3.0 mg/kg) for the capacity to improve PPI in each model. Under vehicle conditions, the prepulse stimuli (75, 80 and 85 dB) inhibited the startle response to a 120 dB auditory stimulus in a graded fashion. Galantamine (depending on dose) improved PPI deficits in all three PPI disruption models, whereas donepezil ameliorated PPI deficits induced by scopolamine and apomorphine, but was not effective in the MK801 model. These results indicate that some AChEIs may have the potential to improve cognition in schizophrenia by improving auditory sensory gating.

KEYWORDS

acetylcholine, acetylcholinesterase, cholinergic, cognition, memory, schizophrenia

INTRODUCTION

Acetylcholinesterase inhibitors (AChEIs) such as donepezil, rivastigmine, and galantamine are commonly used to treat the memory impairments associated with Alzheimer's Disease (AD). This therapeutic approach is based on several large clinical studies of AChEIs in which symptomatic improvements were observed in patients diagnosed with mild to moderate AD (Boada-Rovira et al., 2004; Mintzer and Kershaw, 2003; Raskind et al., 2000; Winblad et al., 2001). There is also increasing evidence that AChEIs may have a therapeutic role for the memory dysfunction associated with a variety of conditions beyond AD, particularly those illnesses that are characterized by presynaptic cholinergic deficits such as Parkinson's Disease, Dementia with Lewy bodies, and Down syndrome (for review see Gustavson and Cummings, 2003).

Interestingly, there is some evidence to suggest that cholinergic function is altered in schizophrenia (thus leading to the question of whether AChEIs might be of benefit in this illness). For example, significant correlations between reduced choline acetyltransferase (i.e., ChAT, the acetylcholine synthesizing enzyme) levels and impaired cognition in schizophrenia have been observed (reviewed, Powchik et al., 1998). Moreover, decreases in both low affinity (α_7) nicotinic acetylcholine receptors (nAChRs) in the hippocampus and high affinity ($\alpha_4\beta_2$) nAChRs in the hippocampus, cortex, striatum, and thalamus have been detected in postmortem brains of schizophrenics (reviewed, Friedman 2004). The number of M_1/M_4 muscarinic acetylcholine receptors (mAChRs) have also been found to be reduced relative to normal postmortem brains in several areas including the hippocampus (Crook et al., 2000), prefrontal cortex (Crook et al., 2001) and striatum (Dean et al., 1996). Likewise,

single photon emission computed tomography (SPECT) in living, unmedicated schizophrenic patients revealed fewer mAChRs in frontal, temporal, and occipital cortex as well as in the striatum and thalamus compared to control subjects (Raedler et al., 2003).

The cholinergic alterations described above may have particular importance to certain features of schizophrenia such as sensory gating abnormalities which are believed to contribute to deficits in attention, cognitive impairment, and possibly hallucinations (Adler et al., 1998). The disruption of "prepulse inhibition" (PPI), a well characterized model for identifying sensory information-processing deficits, is in fact, clearly established in schizophrenia (reviewed, Braff et al., 2001). PPI is defined as the reduction in startle response produced by a low-intensity stimulus presented before a high-intensity, startle-producing stimulus (Graham et al., 1975). Further, PPI is known to be heavily influenced by the hippocampus, thalamus and striatum (reviewed, Swerdlow et al., 2001), i.e., brain regions identified as deficient in cholinergic receptors in schizophrenic patients (see above). The importance of nicotinic-cholinergic function in these brain regions (in particular, in the hippocampus) to PPI has been exemplified in recent clinical studies in which nicotine enhanced tactile PPI in both healthy subjects and schizophrenic patients. The correlational analysis between the enhancement of PPI and the change in neural activation after nicotine (detected by functional magnetic resonance imaging) identified the hippocampus as the primary structure for this modulatory effect of nicotine (Postma et al., 2006)

The measurement of PPI affords many advantages for clinical studies of schizophrenia as well as for the therapeutics of the illness. As summarized by Kumari

and Sharma (2002) impairments in PPI predict poor responses on the Ego Impairment Index-human experience scale, poor performance on the Wisconsin Card Sort Test, and elevated levels of distractibility. The fact that PPI is a cross-species phenomenon also allows for translation between animal experiments and clinical studies (Swerdlow et al., 1999). Accordingly, a number of animal models of PPI impairment have been developed which include neonatal ventral hippocampal lesion, maternal deprivation, isolation rearing, genetic, and pharmacological models, the later being commonly used in antipsychotic drug development studies (Geyer et al., 2001). Such pharmacological models have provided evidence that the neurotransmitters dopamine (Mansbach et al., 1988), glutamate (Mansbach and Geyer, 1989), serotonin (Sipes and Geyer, 1994), and acetylcholine (Jones and Shannon, 2000), are each likely to play an important role in normal sensory gating and PPI as well as disorders of these processes.

Given the established efficacy of AChEIs in the treatment of AD and a variety of animal studies in which cholinergic agonists such as nicotine and cotinine have been observed to enhance PPI (see Terry et al., 2005), we were interested to learn if AChEIs might have the potential to improve PPI. In this study, we compared two commonly used acetylcholinesterase inhibitors, donepezil and galantamine, for ability to improve pharmacologically induced deficits in PPI in rodents using dopamine receptor agonism by apomorphine, NMDA receptor antagonism by MK801, or muscarinic acetylcholine receptor antagonism by scopolamine.

METHODS

Study subjects

One hundred and seventy male albino Wistar rats (2-3 months old) were obtained from Harlan Sprague-Dawley, Inc.) and housed in pairs in a temperature controlled room (25^{O}C), maintained on a 12-hour light/dark cycle with free access to food (Teklad Rodent Diet 8604 pellets, Harlan, Madison, WI) and water. Fifty animals were used in experiments to evaluate the effects of the AChEI's alone on startle amplitude and PPI, 50 rats were used for the galantamine-PPI inhibitor experiments, 50 rats were used for the donepezil-PPI inhibitor experiments, and finally, 20 rats were used to confirm previous (unpublished) results which indicated a lack of significant effect of repeated testing (i.e., up to 4 total exposures to the PPI test) on startle amplitude and PPI. Thus, after habituation to the test apparatus and an initial trial of only startle and prepulse stimuli (described further below), the animals were given one full 60-trial PPI session to establish baseline startle response and PPI levels. After this session, the animals were matched for average startle amplitude (into groups of 8-10) and then assigned to one of the treatment combinations or vehicle-vehicle (see below). Specifically, the groups were assigned to receive one of the treatment combinations and tested a maximum of 3 times after the initial baseline session (when group assignments were made) over an additional 3 week period. Using a balanced crossover design, the AChEI doses and exposure to PPI antagonists were pseudorandomized to obviate any effects associated with the order of drug administration.

All procedures employed during this study were reviewed and approved by the Medical College of Georgia Institutional Animal Care and Use Committee and are consistent with AAALAC guidelines. Measures were taken to minimize pain or discomfort in accordance with the National Institute of Health Guide for the Care and

Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Significant efforts were also made to minimize the total number of animals used while maintaining statistically valid group numbers.

Drugs

All drugs were dissolved in vehicle (0.9% NaCl) and administered in a volume of 1.0 ml/kg. For the dose-effect studies in which the effects of the AChEIs alone on PPI were evaluated, galantamine (Galantamine hydrobromide, Janssen Pharmaceutica, Beerse, Belgium) or donepezil (A&A Pharmachem, Ottawa, Ontario Canada) was injected subcutaneously (s.c.) 30 min before testing followed by vehicle s.c., 10 min before testing. For the apomorphine and MK801 reversal studies, test subjects were administered vehicle or the AChEI s.c., 30 min before testing followed by either vehicle, apomorphine (Sigma A4393) 0.5 mg/kg, or MK-801 (Sigma M-107) 0.1 mg/kg s.c., 10 min before testing. For the scopolamine-reversal studies, scopolamine HBr (Sigma S1875) 0.33 mg/kg was administered 40 min before testing followed by either vehicle or AChEI 20 min before testing. The PPI methods and the compounds used to disrupt PPI (and their doses) were based on earlier studies (Mansbach et al., 1988; Mansbach and Geyer, 1989; Jones and Shannon, 2000b) and recent work in our laboratory (Terry et al., 2005). Galantamine was tested at doses of 0.3, 1.0, and 3.0 mg/kg while donepezil doses were 0.5, 1.0, and 2.0 mg/kg. These doses were based on data obtained in rats in which brain levels of the compounds and the degree of acetylcholinesterase inhibition were compared across a dose and time (Geerts et al., 2005) as well as recent neuropharmacological and behavioral studies in our laboratory (Hernandez et al., 2006).

Behavior testing

All animals were individually handled daily for several minutes for at least one week prior to experimentation. Tests were conducted in four standard startle chambers (San Diego Instruments, San Diego, CA) each consisting of a Plexiglas tube (diameter 8.2 cm, length 25 cm), placed in a sound–attenuated chamber. The tube is mounted on a plastic frame, under which a piezoelectric accelerometer is mounted which records and transduces the motion of the tube. Two days before PPI testing, the experimental subjects were each placed in one of the startle test chambers for a period of 20 minutes (without any startle stimuli) as an initial period of acclimation to the apparatus. One day before drug testing the animals were again placed in the test chamber and then exposed to a 5 min habituation period during which 70 dB background white noise was present. This period was followed by twelve startle stimuli and each prepulse level 3 times (see below). This procedure was conducted to in order to reduce the highly variable responses to the initial exposures to the startle stimuli as well as to ensure that the prepulse stimuli (alone) had no significant effect on the startle response.

On the day of PPI testing, experimental subjects were transported to the startle chamber room and left undisturbed for at least 30 min. Afterwards, the rats were placed in the chamber and then allowed to habituate for a period of 1.0 min. After this period, the rats received 12 startle trials, 12 no-stimulus trials, and 12 trials of each of the prepulse/startle trials (see below) for a total of 60 trials. The intertrial interval ranged from 10 to 30 sec, and the total session lasted about 25-30 min. The startle trials consisted of single 120 dB white noise bursts lasting 20 ms. The PPI trials consisted of a prepulse (20 ms burst of white noise with intensities of 75, 80, or 85 dB) followed 100

ms later by a startle stimulus (120 dB, 20 ms white noise). During the no-stimulus trial, no startle noise was presented, but the movement of the rat was scored. This represented a control trial for detecting differences in overall activity. The different trial types were presented pseudo-randomly, each trial type was presented 12 times, and no two consecutive trials were identical. The resulting movement of the rat in the startle chamber was measured during 100 ms after startle stimulus onset (sampling frequency 1 kHz), rectified, amplified, and fed into a computer that calculated the maximal response over the 100-ms period. Basal startle amplitude was determined as the mean amplitude of the 12 startle trials. PPI was calculated according to the formula 100-100% x (PPx/P120), in which PPx is the mean of the 12 prepulse inhibition trials (i.e., for each individual prepulse intensity), and p120 is the basal startle amplitude. The average level of PPI was also calculated (mean of the responses to pp75, pp80, or pp85) and analyzed separately.

Statistical Analyses

All data were collated and entered into Microsoft Excel spreadsheets. The data were subsequently imported into SigmaStat version 2.03 for statistical analyses. A one or two-way ANOVA (with repeated measures when indicated) was used for all treatment comparisons and the Student-Newman-Keuls method was used for post hoc analyses.

RESULTS

Effects of Repeated PPI Testing

As indicated in the Methods, a series of experiments were conducted to ensure that repeated exposure to PPI testing (i.e., up to 4 total exposures) did not result in

significant changes in startle amplitude or levels of PPI. A group of twenty rats were tested in an initial PPI session, then 10 subjects with similar startle amplitudes and PPI levels were tested once per week for 3 additional sessions (i.e., to mimic the drug study design). As indicated in Fig. 1A, there were highly significant differences in the responses to the various prepulse levels ($F_{(2,18)}$=36.3, p=<0.001) as expected, however, there were no significant effects of the session (i.e., all p values were > 0.05) when the level of PPI or startle amplitude was analyzed (see Fig 1A-C).

Effects of the AChEI's Alone on Startle and PPI

In all of the PPI studies described below, there was a highly significant reduction in the startle response which was dependent upon the magnitude of the prepulse stimulus (i.e., prepulse level difference p<0.001 in all studies—see the open bars in the A insets of Figs. 2.2-7). As indicated in Table 2.1, there were no significant (i.e. p>0.05) effects of galantamine or donepezil on PPI across the doses evaluated nor was there a significant treatment x prepulse level interaction. Similar effects were obtained when the data were averaged across prepulse level (right hand column of Table 2.1). In addition, there were no significant effects of either donepezil or galantamine on the startle amplitude.

Effects of the AChEIs on Pharmacological Inhibitors of PPI

Galantamine

Attenuation of apomorphine - As indicated in Fig. 2A, there were significant differences in responses to the various drug treatments (treatment effect, $F_{(4,42)}$=4.7, p=0.003; the effects of the prepulse levels, $F_{(2,8)}$=88.2, p=<0.001), but the treatment x prepulse level interaction was not significant. Post hoc analyses indicated that

apomorphine (0.5 mg/kg) significantly (p<0.05) diminished PPI at all three prepulse levels when the effect was compared to the vehicle-associated response. Post hoc analyses further indicated that galantamine at the 3.0 mg/kg dose ameliorated the deficits in PPI produced by apomorphine at the 75, 80, and 85 dB prepulse levels. While the 1.0 mg/kg dose of galantamine also ameliorated the deficits induced by apomorphine on PPI at each prepulse level, the effects did not reach the required level of significance (i.e., p>0.05). The positive effect of the 3.0 mg/kg dose of galantamine was also apparent when the data were averaged across the prepulse levels (see Fig. 2.2C). There were no significant effects of apomorphine or the galantamine - apomorphine combination on startle amplitude (Fig. 2.2B).

Attenuation of MK801- As indicated in Fig. 2.3A, there were significant differences in response to the various drug treatments (treatment effect $F_{(4,45)}$=4.4, p=0.004; the effects of the prepulse levels $F_{(2,8)}$=99.4, p<0.001), although the treatment *x* prepulse level interaction was not significant. Post hoc analyses indicated that MK801 (0.1 mg/kg) significantly (p<0.05) diminished PPI (at all prepulse levels) when compared to the vehicle-associated response. Post hoc analysis further indicated that galantamine at a dose of 3.0 mg/kg significantly attenuated the deficits in PPI produced by MK801 at the 85 dB level. This positive effect of galantamine was also apparent when the data were averaged across the prepulse levels (see Fig. 2.3C). There were no significant effects of MK801 or the galantamine -MK801 combination on startle amplitude (Fig. 2.3B).

Attenuation of scopolamine - As indicated in Fig. 2.4A, the treatment responses were significantly different (treatment effect $F_{(4,42)}$=4.1, p=0.007; as were the effects of

the different prepulse levels $F_{(2,8)}$=114.8, p<0.001), although the treatment *x* prepulse level interaction was not significant. Post hoc analyses indicated that scopolamine significantly (p<0.05) diminished PPI (at 75 and 80 dB levels) when compared to the vehicle-associated response. Post hoc analysis further indicated that galantamine at a dose of 1.0 mg/kg improved the deficits in PPI produced by scopolamine at these same two prepulse levels. These positive effects of galantamine were also apparent when the data were averaged across the prepulse levels (see Fig. 2.4C). There were no significant effects of scopolamine or the galantamine -scopolamine combination on startle amplitude (Fig. 2.4B).

Donepezil

Attenuation of apomorphine - As shown in Fig. 2.5A, significant differences were also produced in the donepezil-apomorphine interaction study in response to the various drug treatments (treatment effect, $F_{(4,42)}$=6.4, p<0.001; the effects of the prepulse levels were different, $F_{(2,8)}$=61.6, p<0.001), and the treatment *x* prepulse level interaction was significant, $F_{(88,146)}$=2.1, p=0.043. Post hoc analyses indicated that apomorphine significantly (p<0.05) diminished PPI (at 75 and 80 dB levels) when compared to the vehicle-associated response. Post hoc analysis indicated that donepezil 1.0 mg/kg significantly (p<0.05) improved deficits at these prepulse intensities. Post hoc analysis also confirmed the positive effects of 1.0 mg/kg donepezil when the data were averaged across the prepulse levels (Fig 2.5C). In addition, there were no significant effects of apomorphine or the donepezil-apomorphine combination on startle amplitude (Fig. 2.5B).

Attenuation of MK801-As exhibited in Fig. 2.6A, significant differences were also

observed in the donepezil-MK801 interaction study in response to the various drug treatments (treatment effect, $F_{(4,44)}$=2.9, p=0.031; the effects of the prepulse levels, $F_{(2,8)}$=98.5, p<0.001), and the treatment *x* prepulse level interaction was significant, $F_{(88,146)}$=4.6, p<0.001. Post hoc analyses indicated that MK801 significantly (p<0.05) diminished PPI (at all prepulse levels) when compared to the vehicle-associated response. While there were some trends toward improvements of the MK801-related deficits by donepezil (e.g., the 2.0 mg/kg dose at 85 dB prepulse level) such improvements did not reach significance (i.e., all p values were >0.05). Similarly, there were no significant effects of donepezil on the MK801-associated response when the data were averaged across the prepulse levels (Fig 2.6C). Further, there were no significant effects of MK801 or the donepezil-MK801 combination on startle amplitude (Fig. 2.6B).

Attenuation of scopolamine - Fig 2.7A shows that significant differences were produced in the donepezil-scopolamine interaction study in response to the various drug treatments (treatment effect, $F_{(4,40)}$=2.8, p<0.038; the effects of the prepulse levels were different, $F_{(2,8)}$=80.8, p<0.001), although the treatment *x* prepulse level interaction was not significant. Post hoc analyses indicated that scopolamine significantly (p<0.05) diminished PPI at the 80 dB prepulse level when the effect was compared to the vehicle-associated response. Post hoc analyses further indicated that donepezil at the 1.0 mg/kg dose improved the deficits in PPI produced by scopolamine at this prepulse level. The positive effect of the 1.0 mg/kg dose of donepezil was also apparent when the data were averaged across the prepulse levels (see Fig. 2.7C). Finally, there were no significant effects of scopolamine or the donepezil-scopolamine combination on

startle amplitude (Fig. 2.7B).

DISCUSSION

There were 3 main findings in this study, 1) under vehicle conditions there were no significant effects of repeated exposures to the PPI test method on startle amplitude or PPI in rats for up to 3 sessions (i.e., after the initial test session to establish baselines), 2) neither galantamine nor donepezil had any significant effect on startle amplitude or PPI on their own, 3) galantamine (depending on dose) improved PPI deficits in all three pharmacologic models of PPI impairment, whereas donepezil ameliorated PPI deficits induced by scopolamine and apomorphine, but was not effective in the MK801 model. The lack of benefit of donepezil in the MK801 (PPI) model contrasts with behavioral studies in mice (Csernansky et al., 2005) where donepezil attenuated MK801-induced deficits in fear conditioning as well as spatial and reversal learning better than galantamine. The basis of the differential effects of the compounds observed in our studies and the one cited above is unclear, although there are some differences in the pharmacology of these compounds (i.e., other than that related to AChEI activity) that may be of importance. For example, in culture studies, galantamine, has been observed to potentiate NMDA currents in rat cortical multipolar neurons (Moriguchi et al., 2005). In contrast, donepezil, potentiated NMDA receptors in both bipolar and multipolar neurons, but its effects on multipolar neurons were biphasic. Specifically, donepezil suppressed NMDA-induced currents at moderate concentrations and

potentiated them only at concentrations likely too high to be therapeutically relevant.

In contrast to donepezil, galantamine also functions as a positive allosteric modulator of nAChRs *in vitro* which results in an amplification of the action of the acetylcholine (Maelicke et al., 2001). These nAChR effects of galantamine are interesting in light of reports that cigarette smoking temporarily normalizes sensorimotor gating deficits in schizophrenics (Adler et al., 1993: Kumari et al., 2001), and animal data indicating that chronic nicotine treatment reduced attentional impairments induced by the antipsychotics, haloperidol (Rezvani and Levin, 2004), risperidone, and clozapine (Rezvani et al., 2006). Thus, given the emerging importance of nAChR function in schizophrenia, and the known positive effects of nicotinic agonists on schizophrenic symptoms, the allosteric actions of galantamine at nAChRs could be an important pharmacological advantage.

The observation that both AChEIs improved scopolamine-related decreases in PPI were not particularly surprising since the compounds are well documented to increase synaptic levels of acetylcholine, an effect that would presumably overcome the scopolamine antagonism at muscarinic receptors as well as increase tone at unblocked nicotinic receptors. The experiments were conducted, however, to confirm that this pharmacological action (i.e., improved cholinergic function) indeed likely contributed to the effects of these compounds on PPI. Other animal data that support such a premise are evident in a recent study in which PPI deficits induced by immunolesions of cholinergic neurons of the nucleus basalis were reversed by the AChEI, rivastigmine (Ballmaier et al., 2002). Additional data to support the role of muscarinic receptors in PPI (and that these receptors could serve as targets for drug development in

schizophrenia) have been reported in a recent study in which, xanomeline, an M_1/M_4 AChR agonist (Jones et al., 2005; Stanhope et al., 2001), normalized apomorphine-reduced PPI levels.

The data described in this report thus support the hypothesis that AChEIs might have the potential to serve as therapeutic options for schizophrenia by improving PPI and cognitive function. To date, however, the data collected in the relatively small number of clinical studies that have been designed to evaluate the potential cognitive benefits of AChEIs in schizophrenia have been equivocal. For example, the beneficial effects of donepezil or rivastigmine on cognition in schizophrenics as add-on treatments to antipsychotics observed in small preliminary investigations, open-label studies and case reports (e.g., Buchanan et al., 2003; Stryjer et al., 2003; Lenzi et al., 2003) were not confirmed in larger randomized, double-blind, and placebo controlled studies (see Friedman et al., 2002; Freudenreich et al., 2005; Sharma et al., 2006). In the case of galantamine, there are also several small studies that indicate a potential benefit to schizophrenic patients including a recent randomized, double-blind clinical trial (N=8) where the AChEI improved short-term memory and attention in schizophrenic or schizoaffective patients who were stabilized on risperidone (Schubert et al., 2006). While such data are encouraging, much larger studies will be required to verify the validity of this approach as a reliable therapeutic intervention in schizophrenia.

As in the case of schizophrenia, there are several small clinical studies that would support the use of AChEIs as adjunctive therapy in other psychiatric and neurological disorders where PPI deficits have been reported. For example, donepezil and rivastigmine have been observed to improve several autistic behaviors in children

(Hardan and Handen, 2002; Chez et al., 2004), while in autistic adults, adjunctive galantamine therapy enhanced language and communication skills (Hertzman, 2003). In addition, rivastigmine and galantamine have been observed to improve cognitive function as well as hallucinations in Parkinson's disease patients (Bullock and Cameron, 2002; Aarsland et al., 2003) and further, rivastigmine has been reported to ameliorate cognitive deficits and slow motor deterioration in patients with Huntington's Disease (de Tommaso et al., 2004).

In summary, the results of this rodent study indicate that the clinically used AChEIs, galantamine and donepezil, have the ability to improve auditory sensory gating in established pharmacologic models of impaired PPI. These data combined with the positive clinical data described above suggest that efforts to increase cholinergic activity (i.e., by cholinesterase inhibitors or other means) in the brain warrant further investigation as potential therapeutic options for schizophrenia and other conditions where PPI is disrupted.

AKNOWLEDGEMENTS

This work was supported in part by the American Foundation for Pharmaceutical Education's Pre-doctoral Fellowship program and by the National Institute of Mental Health (MH 066233 to AVT).

REFERENCES

Aarsland, D., Hutchinson, M., Larsen, J.P. 2003. Cognitive, psychiatric and motor response to galantamine in Parkinson's disease with dementia. *Int J Geriatr Psychiatry. 18*(10), 937-941.

Adler, L.E., Hoffer, L.D., Wiser, A., Freedman, R. 1993. Normalization of auditory physiology by cigarette smoking in schizophrenic patients. *Am J Psychiatry 150*(12), 1856-1861.

Adler L.E., Olincy A., Waldo M., Harris J.G., Griffith J., Stevens K., Flach K., Nagamoto H., Bickford P., Leonard S., Freedman R. 1998. Schizophrenia, sensory gating, and nicotinic receptors. *Schizophr Bull* 24:189-202.

Ballmaier, M., Casamenti, F., Scali, C., Mazzoncini, R., Zoli, M., Pepeu, G., et al. 2002. Rivastigmine antagonizes deficits in prepulse inhibition induced by selective immunolesioning of cholinergic neurons in nucleus basalis magnocellularis. *Neuroscience 114*(1), 91.

Boada-Rovira, M., Brodaty, H., Cras, P., Baloyannis, S., Emre, M., Zhang, R., et al. 2004. Efficacy and safety of donepezil in patients with Alzheimer's disease: results of a global, multinational, clinical experience study. *Drugs Aging 21*(1), 43-53.

Braff D.L., Geyer M.A., Swerdlow N.R., 2001. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology* 156:234-258.

Buchanan, R.W., Summerfelt, A., Tek, C., Gold, J. 2003. An open-labeled trial of adjunctive donepezil for cognitive impairments in patients with schizophrenia. *Schizophrenia Research 59*(1), 29.

Bullock, R., Cameron, A. 2002. Rivastigmine for the treatment of dementia and visual hallucinations associated with Parkinson's disease: a case series. *Curr Med Res Opin 18*(5), 258-264.

Chez, M.G., Aimonovitch, M., Buchanan, T., Mrazek, S., Tremb, R.J. 2004. Treating autistic spectrum disorders in children: utility of the cholinesterase inhibitor rivastigmine tartrate. *J Child Neurol. 19*(3), 165-169.

Crook, J.M., Tomaskovic-Crook, E., Copolov, D.L., Dean, B. 2000. Decreased muscarinic receptor binding in subjects with schizophrenia: a study of the human hippocampal formation. *Biol Psychiatry 48*(5), 381-388.

Crook, J.M., Tomaskovic-Crook, E., Copolov, D.L., Dean, B. 2001. Low muscarinic receptor binding in prefrontal cortex from subjects with schizophrenia: a study of

Brodmann's areas 8, 9, 10, and 46 and the effects of neuroleptic drug treatment. *Am J Psychiatry 158*(6), 918-925.

Csernansky, J.G., Martin, M., Shah, R., Bertchume, A., Colvin, J., Dong, H. 2005. Cholinesterase inhibitors ameliorate behavioral deficits induced by MK-801 in mice. *Neuropsychopharmacology. 30*(12), 2135-2143.

de Tommaso, M., Specchio, N., Sciruicchio, V., Difruscolo, O., Specchio, L.M. 2004. Effects of rivastigmine on motor and cognitive impairment in Huntington's disease. *Mov Disord 19*(12), 1516-1518.

Dean, B., Crook, J.M., Opeskin, K., Hill, C., Keks, N., Copolov, D.L. 1996. The density of muscarinic M1 receptors is decreased in the caudate-putamen of subjects with schizophrenia. *Mol Psychiatry 1*(1), 54-58.

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 J.I. 2004 Cholinergic targets for cognitive enhancement in schizophrenia: focus on cholinesterase inhibitors and muscarinic agonists. *Psychopharmacology* (Berl) 174:45-53..

Freudenreich, O., Herz, L., Deckersbach, T., Evins, A.E., Henderson, D.C., Cather, C., et al. 2005. Added donepezil for stable schizophrenia: a double-blind, placebo-controlled trial. *Psychopharmacology (Berl). 181*(2), 358-363. Epub 2005 Oct 2014.

Geerts, H., Guillaumat, P.-O., Grantham, C., Bode, W., Anciaux, K., Sachak, S. 2005. Brain levels and acetylcholinesterase inhibition with galantamine and donepezil in rats, mice, and rabbits. *Brain Research 1033*(2), 186.

Geyer, M.A., Krebs-Thomson, K., Braff, D.L., Swerdlow, N.R. 2001. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology (Berl) 156*(2-3), 117-154.

Graham, F.K., Putnam, L.E., Leavitt, L.A. 1975. Lead-stimulation effects of human cardiac orienting and blink reflexes. *J Exp Psychol Hum Percept Perform 104*(2), 175-182.

Gustavson, A.R., Cummings, J.L. 2003. Cholinesterase inhibitors in non-Alzheimer dementias. *J Am Med Dir Assoc 4*(6 Suppl), S165-169.

Hardan, A.Y., Handen, B.L. 2002. A retrospective open trial of adjunctive donepezil in children and adolescents with autistic disorder. *J Child Adolesc Psychopharmacol 12*(3), 237-241.

Hernandez C.M., Gearhart D.A., Parikh V., Hohnadel E.J., Davis L.W., Middlemore M.L., Warsi S., Waller J.L., and Terry, A.V., Jr. 2006. Comparison of galantamine and donepezil for effects on nerve growth factor, cholinergic markers, and memory performance in aged rats. *J Pharmacol Exp Ther* 316:679-694.

Hertzman, M. 2003. Galantamine in the treatment of adult autism: a report of three clinical cases. *Int J Psychiatry Med 33*(4), 395-398.

Jones, C.K., Eberle, E.L., Shaw, D.B., McKinzie, D.L., Shannon, H.E. 2005. Pharmacologic interactions between the muscarinic cholinergic and dopaminergic systems in the modulation of prepulse inhibition in rats. *J Pharmacol Exp Ther 312*(3), 1055-1063.

Jones, C.K., Shannon, H.E. 2000. Muscarinic cholinergic modulation of prepulse inhibition of the acoustic startle reflex. *J Pharmacol Exp Ther 294*(3), 1017-1023.

Kumari, V., Soni, W., Sharma, T. 2001. Influence of cigarette smoking on prepulse inhibition of the acoustic startle response in schizophrenia. *Hum Psychopharmacol. 16*(4), 321-326.

Kumari V, Sharma T. 2002. Effects of typical and atypical antipsychotics on prepulse inhibition in schizophrenia: a critical evaluation of current evidence and directions for future research. *Psychopharmacology* 162:97-101.

Lenzi, A., Maltinti, E., Poggi, E., Fabrizio, L., Coli, E. 2003. Effects of rivastigmine on cognitive function and quality of life in patients with schizophrenia. *Clin Neuropharmacol 26*(6), 317-321.

Maelicke, A., Samochocki, M., Jostock, R., Fehrenbacher, A., Ludwig, J., Albuquerque, E.X., et al. 2001. Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease. *Biol Psychiatry 49*(3), 279-288.

Mansbach, R.S., Geyer, M.A. 1989. Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. *Neuropsychopharmacology. 2*(4), 299-308.

Mansbach, R.S., Geyer, M.A., Braff, D.L. 1988. Dopaminergic stimulation disrupts sensorimotor gating in the rat. *Psychopharmacology (Berl). 94*(4), 507-514.

Mintzer, J.E., Kershaw, P. 2003. The efficacy of galantamine in the treatment of Alzheimer's disease: comparison of patients previously treated with acetylcholinesterase inhibitors to patients with no prior exposure. *Int J Geriatr Psychiatry 18*(4), 292-297.

Moriguchi, S., Zhao, X., Marszalec, W., Yeh, J.Z., Narahashi, T. 2005. Modulation of N-Methyl-D-aspartate receptors by donepezil in rat cortical neurons. *J Pharmacol Exp*

Ther 315(1), 125-135.

Postma, P., Gray, J.A., Sharma, T., Geyer, M., Mehrotra, R., Das, M., et al. 2006. A behavioural and functional neuroimaging investigation into the effects of nicotine on sensorimotor gating in healthy subjects and persons with schizophrenia. *Psychopharmacology (Berl). 184*(3-4), 589-599. Epub 2006 Feb 2002.

Powchik, P., Davidson, M., Haroutunian, V., Gabriel, S.M., Purohit, D.P., Perl, D.P., et al. 1998. Postmortem studies in schizophrenia. *Schizophr Bull 24*(3), 325-341.

Raedler, T.J., Knable, M.B., Jones, D.W., Urbina, R.A., Egan, M.F., Weinberger, D.R. 2003. Central muscarinic acetylcholine receptor availability in patients treated with clozapine. *Neuropsychopharmacology 28*(8), 1531-1537.

Raskind, M.A., Peskind, E.R., Wessel, T., Yuan, W. 2000. Galantamine in AD: A 6-month randomized, placebo-controlled trial with a 6-month extension. The Galantamine USA-1 Study Group. *Neurology 54*(12), 2261-2268.

Rezvani, A.H., Caldwell, D.P., Levin, E.D. 2006. Chronic nicotine interactions with clozapine and risperidone and attentional function in rats. *Prog Neuropsychopharmacol Biol Psychiatry. 30*, 190-197.

Rezvani, A.H., Levin, E.D. 2004. Nicotine-antipsychotic drug interactions and attentional performance in female rats. *Eur J Pharmacol. 486*(2), 175-182.

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, 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.

Sipes, T.A., Geyer, M.A. 1994. Multiple serotonin receptor subtypes modulate prepulse inhibition of the startle response in rats. *Neuropharmacology. 33*(3-4), 441-448.

Stanhope, K.J., Mirza, N.R., Bickerdike, M.J., Bright, J.L., Harrington, N.R., Hesselink, M.B., et al. 2001. The muscarinic receptor agonist xanomeline has an antipsychotic-like profile in the rat. *J Pharmacol Exp Ther 299*(2), 782-792.

Stryjer, R., Strous, R.D., Bar, F., Werber, E., Shaked, G., Buhiri, Y., et al. 2003. Beneficial effect of donepezil augmentation for the management of comorbid schizophrenia and dementia. *Clin Neuropharmacol. 26*(1), 12-17.

Swerdlow, N.R., Braff, D.L., Geyer, M.A. 1999. Cross-species studies of sensorimotor gating of the startle reflex. *Ann NY Acad Sci 877*(1), 202-216.

Swerdlow NR, Geyer MA, Braff DL. 2001. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology* 156(2-3):194-215.

Terry, A.V., Jr., Hernandez, C.M., Hohnadel, E.J., Bouchard, K.P., Buccafusco, J.J. 2005. Cotinine, a neuroactive metabolite of nicotine: potential for treating disorders of impaired cognition. *CNS Drug Rev 11*(3), 229-252.

Winblad, B., Engedal, K., Soininen, H., Verhey, F., Waldemar, G., Wimo, A., et al. 2001. A 1-year, randomized, placebo-controlled study of donepezil in patients with mild to moderate AD. *Neurology 57*(3), 489-495.

Table 2.1 AChEI Effects on Startle Response and PPI

			% PPI ± SEM			
Group	**Startle (AU)**	**Nulstim**	**75 dB**	**80 dB**	**85 dB**	**Mean**
VEH	776.1 ± 36.8	16.7 ± 2.4	51.3 ± 3.2	64.0± 3.8	81.2 ± 2.4	65.5 ± 2.4
0.5 DON	811.7 ± 258.6	21.2 ± 3.7	56.0 ± 8.7	66.2 ± 6.7	78.5 ± 4.6	66.9 ± 6.4
1.0 DON	489.5 ± 164.0	24.9 ± 4.1	57.0 ± 6.5	69.0 ± 5.5	75.8 ± 5.5	67.3 ± 5.2
2.0 DON	623.1 ± 142.0	13.0 ± 2.8	63.2 ± 3.0	70.8 ± 4.0	83.4 ± 3.1	72.4 ± 3.0
0.3 GAL	608.2 ± 93.8	17.7 ± 1.9	44.6 ± 6.8	61.7 ± 5.2	68.0 ± 6.1	58.1 ± 6.2
1.0 GAL	760.7 ± 221.7	21.3 ± 3.1	57.2 ± 5.8	69.6 ± 5.3	76.6 ± 4.4	67.8 ± 1.6
3.0 GAL	582.5 ± 204.3	15.2 ± 2.6	49.9 ± 8.7	60.2 ± 6.3	71.0 ± 5.7	59.6 ± 6.8

Figure Legends

Fig 2.1 Effects of repeated exposures to the PPI testing method in rats. **(A)** Level of prepulse inhibition associated with three prepulse intensities (75, 80, and 85 dB). **(B)** Startle amplitude. **(C)** Level of prepulse inhibition averaged across prepulse level. Bars represent mean ± S.E.M. for each session (N=10).

Fig 2.2 (A) Effects of apomorphine (0.5 mg/kg) and several doses of galantamine on apomorphine-induced deficits in prepulse inhibition in rats associated with three prepulse intensities (75, 80, and 85 dB). **(B)** Effects of apomorphine and galantamine combined with apomorphine on startle amplitude. **(C)** Effects of apomorphine (0.5 mg/kg) and several doses of galantamine on apomorphine-induced deficits in prepulse inhibition averaged across prepulse level. Bars represent mean ± S.E.M. for each treatment (N=8-10). VEH = vehicle; GAL = galantamine; APO = apomorphine. * = significantly different ($p<0.05$) than the vehicle associated response. + = significantly different ($p<0.05$) than the apomorphine-associated response.

Fig 2.3 (A) Effects of MK801 (0.1 mg/kg) and several doses of galantamine on MK-801-induced deficits in prepulse inhibition in rats associated with three prepulse intensities (75, 80, and 85 dB). **(B)** Effects of MK801 and galantamine combined with MK801 on startle amplitude. **(C)** Effects of galantamine on MK801-induced deficits in prepulse inhibition averaged across prepulse level. Bars represent mean ± S.E.M. for each treatment (N=10). VEH = vehicle; GAL = galantamine. * = significantly different ($p<0.05$) than the vehicle associated response. + = significantly different ($p<0.05$) than the MK801-associated response.

Fig 2.4 (A) Effects of scopolamine (0.33 mg/kg) and several doses of galantamine on scopolamine-induced deficits in prepulse inhibition in rats associated with three prepulse intensities (75, 80, and 85 dB). **(B)** Effects of scopolamine and galantamine combined with scopolamine on startle amplitude. **(C)** Effects of galantamine on scopolamine-induced deficits in prepulse inhibition across prepulse level. Bars represent mean ± S.E.M. for each treatment (N=8-10). VEH = vehicle; SCOP = scopolamine; DON= donepezil. * = significantly different ($p<0.05$) than the vehicle associated response. + = significantly different ($p<0.05$) than the scopolamine-associated response.

Fig 2.5 (A) Effects of apomorphine (0.5 mg/kg) and several doses of donepezil on apomorphine-induced deficits in prepulse inhibition in rats associated with three prepulse intensities (75, 80, and 85 dB). **(B)** Effects of apomorphine and donepezil combined with apomorphine on startle amplitude. **(C)** Effects of apomorphine (0.5 mg/kg) and several doses of donepezil on apomorphine-induced deficits in prepulse inhibition averaged across prepulse level. Bars represent mean ± S.E.M. for each treatment (N=8-10). VEH = vehicle; DON = donepezil; APO = apomorphine. * = significantly different ($p<0.05$) than the vehicle associated response. + = significantly different ($p<0.05$) than the apomorphine-associated response.

Fig 2.6 (A) Effects of MK801 (0.1 mg/kg) and several doses of donepeizil on MK-801-induced deficits in prepulse inhibition in rats associated with three prepulse intensities

(75, 80, and 85 dB). **(B)** Effects of MK801 and donepezil combined with MK801 on startle amplitude. **(C)** Effects of donepezil on MK801-induced deficits in prepulse inhibition averaged across prepulse level. Bars represent mean ± S.E.M. for each treatment (N=9-10). VEH = vehicle; DON = donepezil. * = significantly different ($p<0.05$) than the vehicle associated response. + = significantly different ($p<0.05$) than the MK801-associated response.

Fig 2.7 (A) Effects of scopolamine (0.33 mg/kg) and several doses of donepezil on scopolamine-induced deficits in prepulse inhibition in rats associated with three prepulse intensities (75, 80, and 85 dB). **(B)** Effects of scopolamine and donepezil combined with scopolamine on startle amplitude. **(C)** Effects of donepezil on scopolamine-induced deficits in prepulse inhibition across prepulse level. Bars represent mean ± S.E.M. for each treatment (N=8-10). VEH = vehicle; SCOP = scopolamine; DON= donepezil. * = significantly different ($p<0.05$) than the vehicle associated response. + = significantly different ($p<0.05$) than the scopolamine-associated response.

Fig 2.1

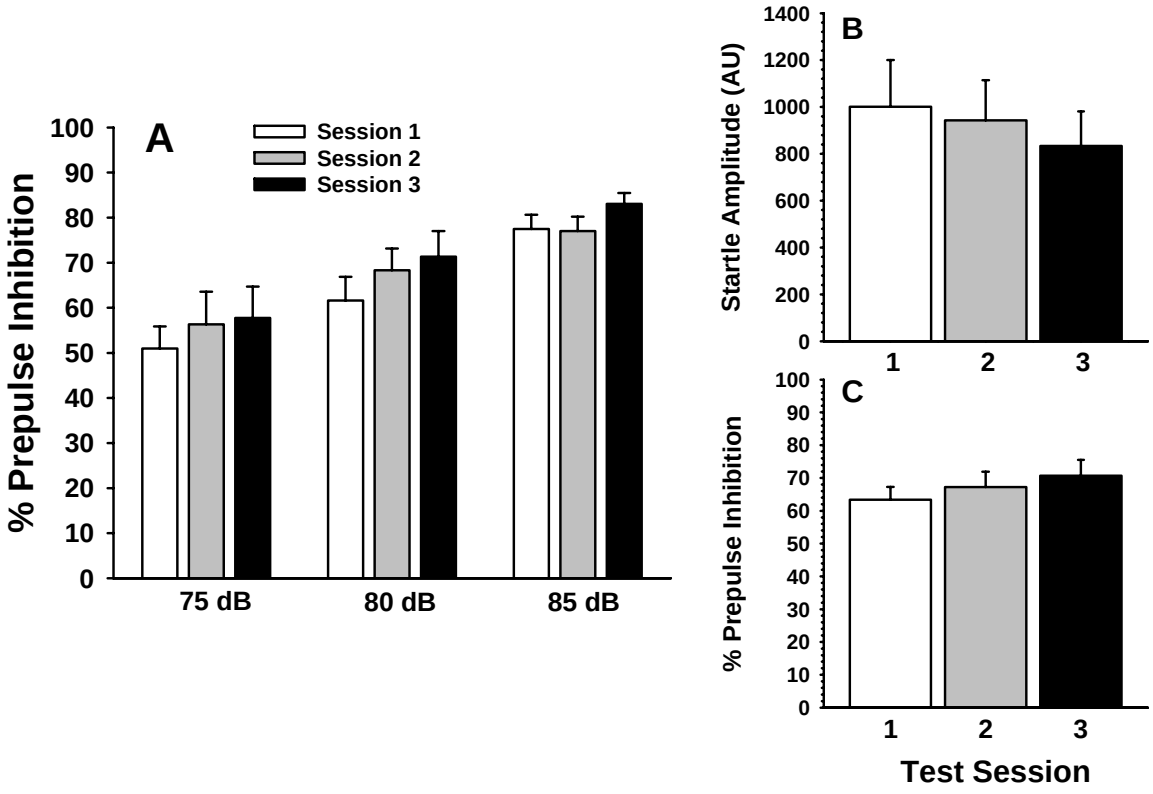

A
Session 1
Session 2
Session 3
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
75 dB
80 dB
85 dB
B
Startle Amplitude (AU)
1400
1200
1000
800
600
400
200
0
1
2
3
C
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
1
2
3
Test Session

Fig 2.2

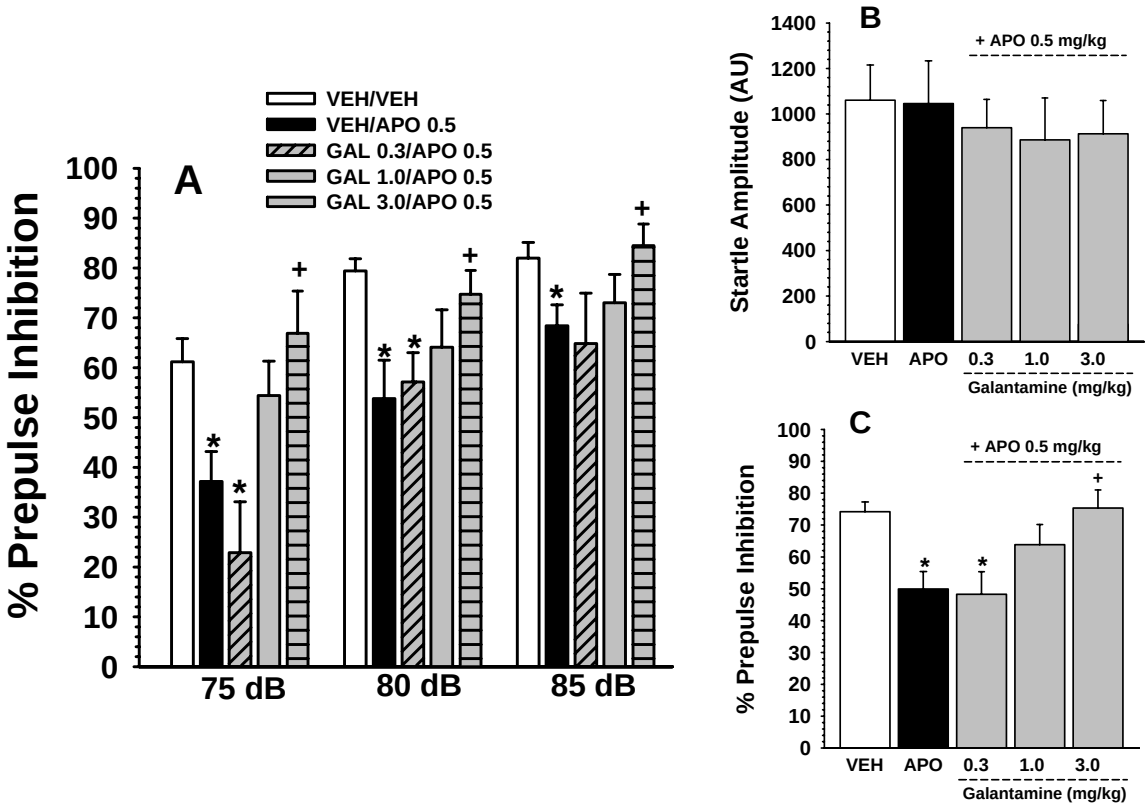

A
VEH/VEH
VEH/APO 0.5
GAL 0.3/APO 0.5
GAL 1.0/APO 0.5
GAL 3.0/APO 0.5
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
75 dB
80 dB
85 dB
B
+ APO 0.5 mg/kg
Startle Amplitude (AU)
1400
1200
1000
800
600
400
200
0
VEH
APO
0.3
1.0
3.0
Galantamine (mg/kg)
C
+ APO 0.5 mg/kg
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
VEH
APO
0.3
1.0
3.0
Galantamine (mg/kg)

Fig 2.3

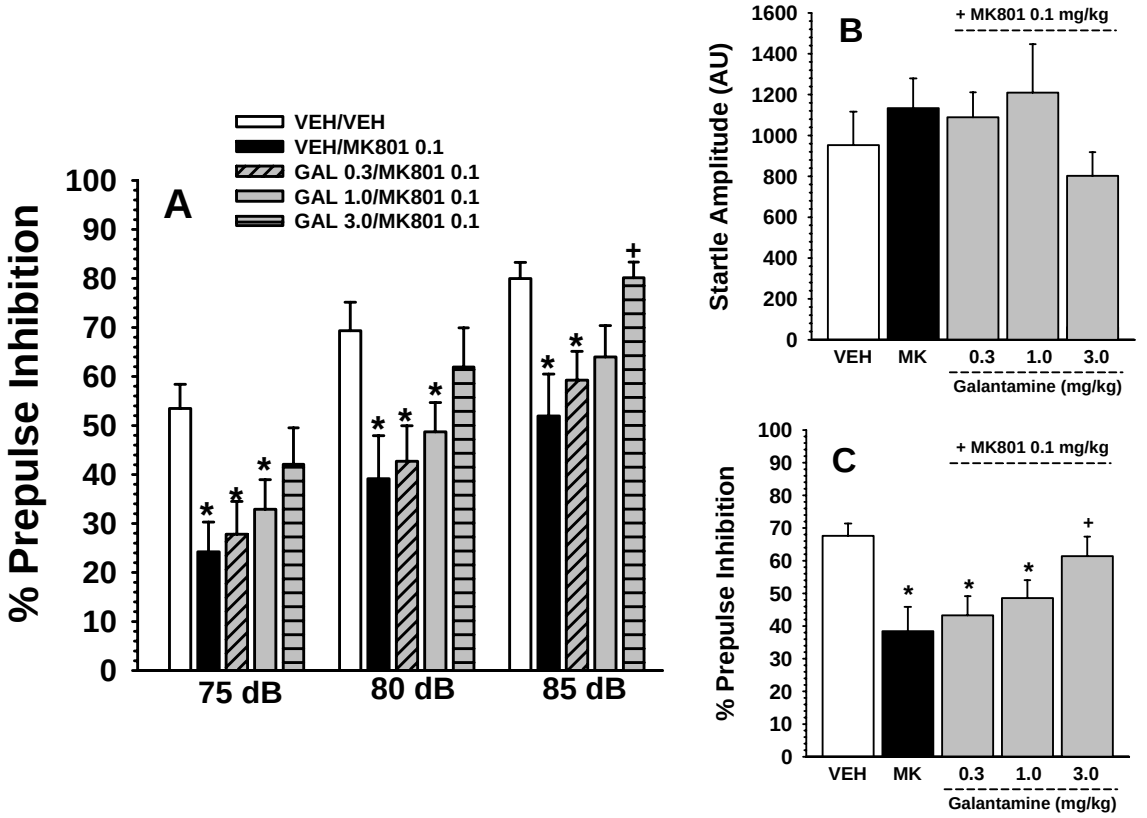

A
VEH/VEH
VEH/MK801 0.1
GAL 0.3/MK801 0.1
GAL 1.0/MK801 0.1
GAL 3.0/MK801 0.1
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
75 dB
80 dB
85 dB
B
+ MK801 0.1 mg/kg
Startle Amplitude (AU)
1600
1400
1200
1000
800
600
400
200
0
VEH
MK
0.3
1.0
3.0
Galantamine (mg/kg)
C
+ MK801 0.1 mg/kg
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
VEH
MK
0.3
1.0
3.0
Galantamine (mg/kg)

Fig 2.4

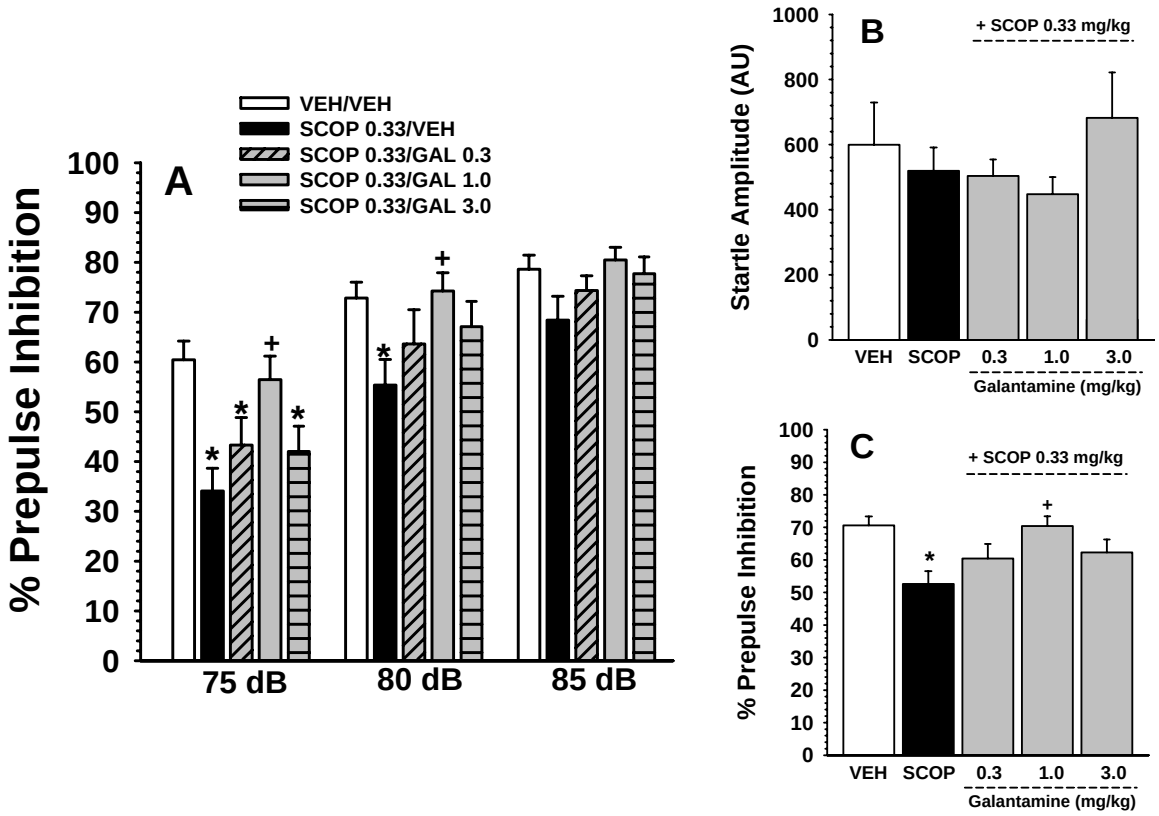

A
VEH/VEH
SCOP 0.33/VEH
SCOP 0.33/GAL 0.3
SCOP 0.33/GAL 1.0
SCOP 0.33/GAL 3.0
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
75 dB
80 dB
85 dB
B
+ SCOP 0.33 mg/kg
Startle Amplitude (AU)
1000
800
600
400
200
0
VEH
SCOP
0.3
1.0
3.0
Galantamine (mg/kg)
C
+ SCOP 0.33 mg/kg
% Prepulse Inhibition
VEH
SCOP
0.3
1.0
3.0
Galantamine (mg/kg)

Fig 2.5

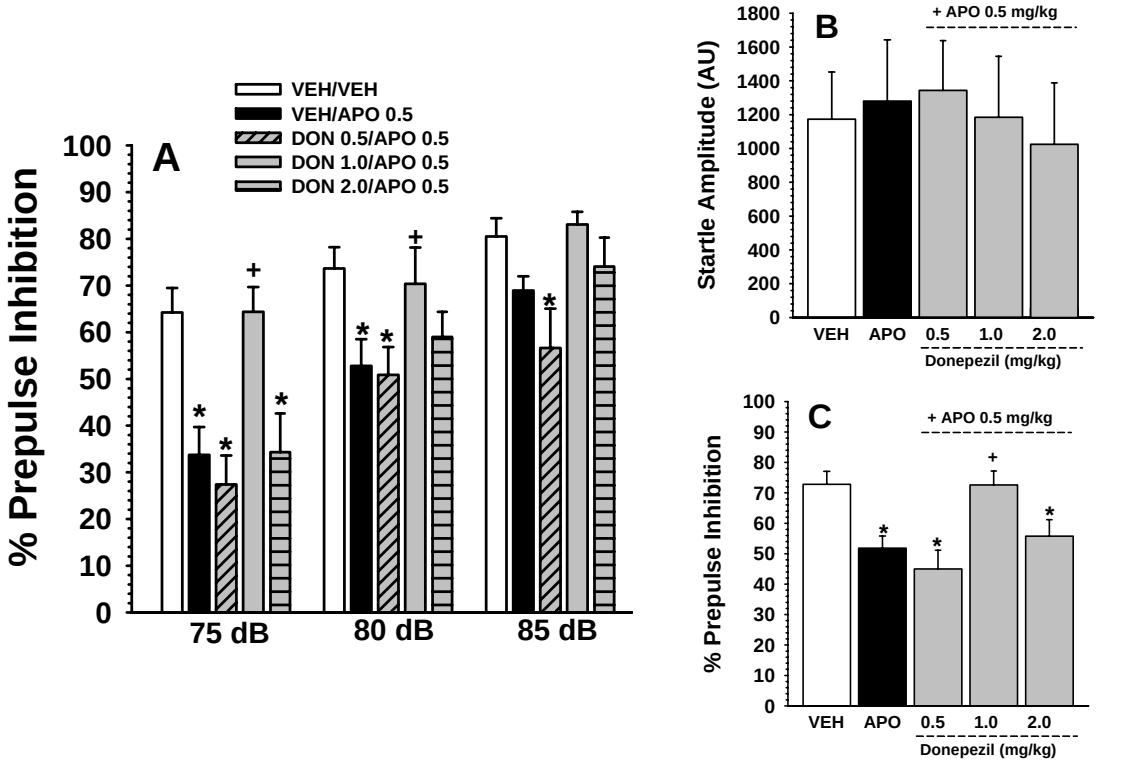

A
VEH/VEH
VEH/APO 0.5
DON 0.5/APO 0.5
DON 1.0/APO 0.5
DON 2.0/APO 0.5
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
75 dB
80 dB
85 dB
B
+ APO 0.5 mg/kg
Startle Amplitude (AU)
1800
1600
1400
1200
1000
800
600
400
200
0
VEH
APO
0.5
1.0
2.0
Donepezil (mg/kg)
C
+ APO 0.5 mg/kg
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
VEH
APO
0.5
1.0
2.0
Donepezil (mg/kg)

Fig 2.6

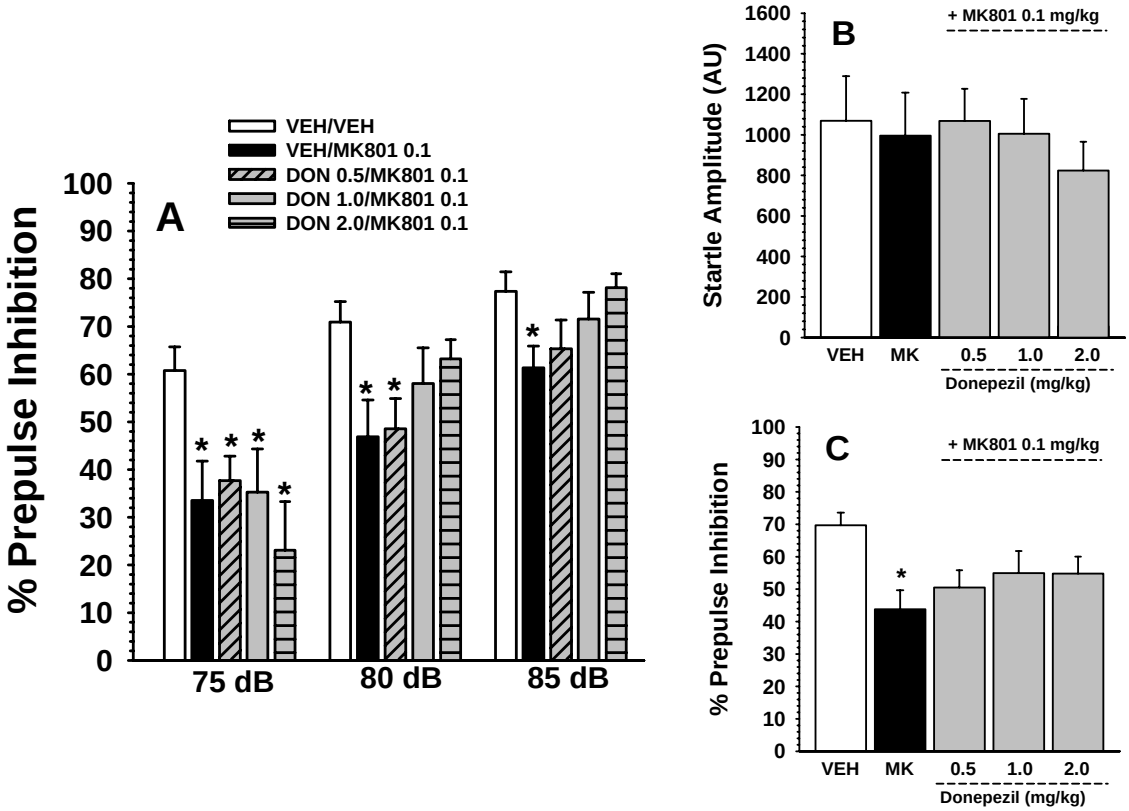
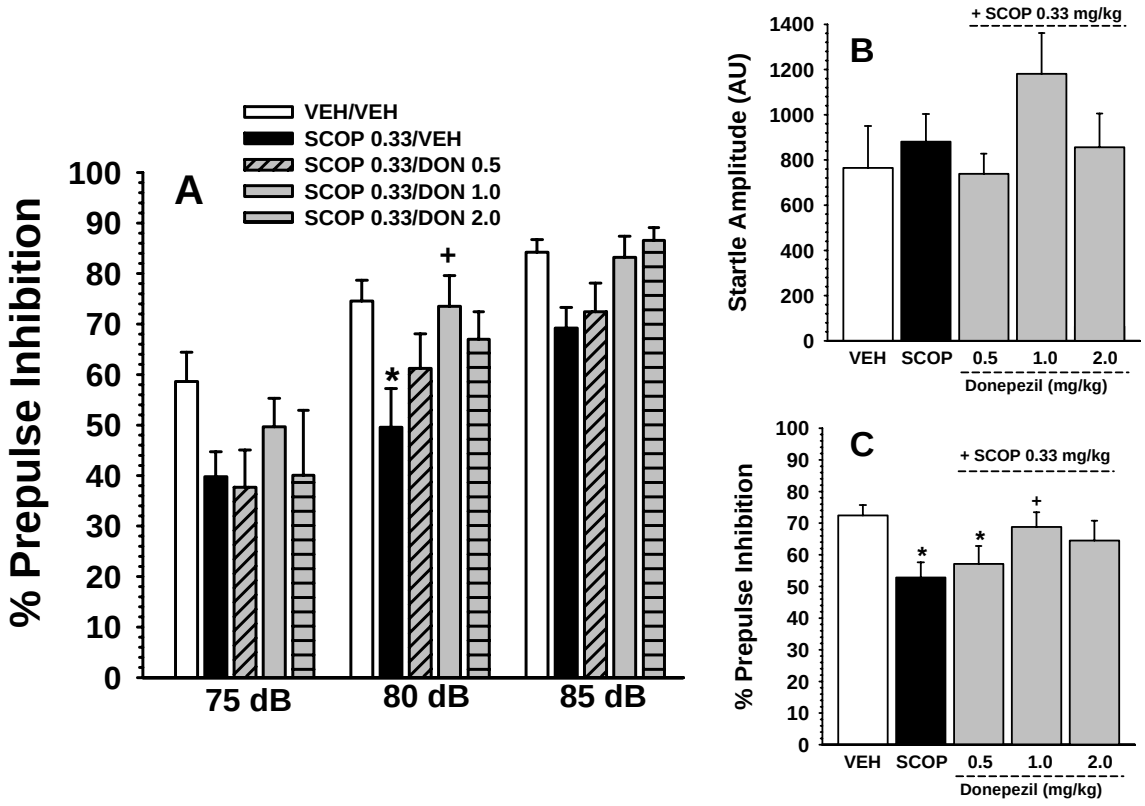

A
VEH/VEH
VEH/MK801 0.1
DON 0.5/MK801 0.1
DON 1.0/MK801 0.1
DON 2.0/MK801 0.1
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
75 dB
80 dB
85 dB
B
+ MK801 0.1 mg/kg
Startle Amplitude (AU)
1600
1400
1200
1000
800
600
400
200
0
VEH
MK
0.5
1.0
2.0
Donepezil (mg/kg)
C
+ MK801 0.1 mg/kg
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
VEH
MK
0.5
1.0
2.0
Donepezil (mg/kg)

Fig 2.7


A
VEH/VEH
SCOP 0.33/VEH
SCOP 0.33/DON 0.5
SCOP 0.33/DON 1.0
SCOP 0.33/DON 2.0
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
75 dB
80 dB
85 dB
*
+
B
+ SCOP 0.33 mg/kg
Startle Amplitude (AU)
1400
1200
1000
800
600
400
200
0
VEH
SCOP
0.5
1.0
2.0
Donepezil (mg/kg)
C
+ SCOP 0.33 mg/kg
% Prepulse Inhibition
100
90
80
70
60
50
40
30
20
10
0
VEH
SCOP
0.5
1.0
2.0
Donepezil (mg/kg)
*
*
+

CHAPTER 3

SUBCHRONIC HALOPERIDOL AND RISPERIDONE TREATMENT DIFFERENTIALLY AFFECTS RADIAL ARM AND WATER MAZE PERFORMANCE IN RATS

Hohnadel, E., and A.V. Terry, Jr. To be submitted to Neuropharmacology.

ABSTRACT

Clinicians and researchers are searching for the most effective yet cost efficient treatment strategies for improving positive, negative, and cognitive symptoms of schizophrenia while resulting in the least adverse effects. Chronic drug delivery, compared to acute, in animal studies more closely parallels clinical schizophrenia treatment and, thus, effects on true patient populations. In this study, rats were administered either the first generation antipsychotic haloperidol (1.0 or 2.0 mg/kg/day) or the second generation antipsychotic, risperidone (1.25 or 2.5 mg/kg/day) in drinking water (or vehicle). At day 15 of drug exposure, subjects were tested in either a radial arm maze, win-shift acquisition task followed by a delayed non-match to position (DNMTP) procedure, or a version of the Morris water maze which included acquisition, recall, and extinction trials. In radial arm maze experiments neither haloperidol nor risperidone affected win-shift acquisition or DNMTP performance. However, haloperidol, but not risperidone, impaired water maze hidden platform acquisition in a dose-dependent manner as indicated by increased latencies and distances swam. Further, probe trial performance was impaired by haloperidol (but not risperidone). Risperidone was without significant effect in the subsequent extinction trials. Due to impairments in the first probe trial, it was not possible to fully assess the effect of haloperidol on extinction. Additional analyses indicated elevated levels of thigmotaxis and reduced swim speeds in the haloperidol-treated animals possibly indicating psychomotor impairments and elevated anxiety. To further explore the possibility that haloperidol effects in the water maze might be influenced by increased anxiety levels, elevated plus maze and light dark preference tests were conducted. In

these measures, anxiety levels were indeed elevated in animals treated with the 2.0 mg/kg/day dose of haloperidol. The results of this study indicate that subchronic exposure to therapeutic doses of haloperidol (but not risperidone) leads to impairments in the performance of a spatial reference learning procedure. These deficits may in part be due to haloperidol-related psychomotor impairments and elevated levels of anxiety.

INTRODUCTION

Cognitive deficits have moved to the forefront of drug targeting in schizophrenia. These deficits actually dictate the severity of the disorder as well as predict functional outcome (reviewed (Green, 2006; Green, 1996; reviewed Kurtz, 2006; Kurtz et al., 2005; Pinkham and Penn, 2006; Villalta-Gil et al., 2006). While current therapies for schizophrenia effectively control positive symptoms and to a lesser extent the negative symptoms, they only provide modest improvements of the debilitating cognitive deficits (Keefe et al., 2007). Further, considering the fact that the longest clinical studies conducted to date only followed patients for a maximum of two years (Schooler et al., 2005), most clinical studies that report improvements in cognition have probably been too short to make this conclusion.

The primary therapies for schizophrenia, first generation antipsychotics (FGAs), also known as typical or conventional antipsychotics, are potent D_2 dopamine receptor antagonists. Due to the high incidence of extrapyramidal symptoms (EPS), FGAs have mostly been replaced in the US and other developed countries by atypical or second generation antipsychotics (SGAs). While most SGAs have antagonist activity at D_2 receptors as well, they also act as antagonists at serotonin receptors ($5HT_{2A}$). This additional mechanism has been hypothesized to decrease extrapyramidal symptoms (Kessler et al., 2005; reviewed by Seeman, 2002) and potentially result in a more favorable effect on cognition. However, weight gain, hyperlipidemia, and diabetes mellitus (reviewed Shirzadi and Ghaemi, 2006)) have emerged as significant side effects associated with SGAs. These more recent findings have researchers and clinicians reconsidering the choice of SGAs as first-line therapy over FGAs. This debate

has been further fueled by the focus on cognitive improvement (Green et al., 2002; Keefe et al., 2004; Keefe et al., 2007; McCue et al. 2006), the cost effectiveness of treatment with SGAs versus FGAs (Polsky et al., 2006), and weaknesses of earlier comparative studies in which large doses of haloperidol were used (Keefe et al., 2004; Crespo-Facorro et al., 2006; Sanger et al., 1999) or results confounded by concomitant medications.

Only within animal studies can complications of drug use/abuse, drop out rates, polypharmacy, life style factors, and noncompliance be eliminated as confounders to the effects of the antipsychotic drugs themselves. Furthermore, chronic drug delivery (as opposed to acute administration) in animal studies, more closely reflects the clinical therapeutics of schizophrenia. 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, and nicotinic (alpha(7)) and muscarinic (M(2)) acetylcholine receptors. 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, our work supports the argument 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 (Terry et al., 2007a). In behavioral studies, haloperidol impaired performance in object recognition and Morris water maze tasks as early as 8-14 days whereas risperidone-treated animals showed slight improvements in Morris water maze early during treatment but were impaired in the maze at the longest time point, 174-180

days (Terry et al., 2007a). In another study involving 45-180 days of treatment, animals receiving haloperidol displayed temporally-dependent behavioral deficits in the Morris water maze even after a washout period of 7 days before testing was initiated. In contrast, even after 90 days of treatment, no differences were observed among animals treated with vehicle, haloperidol, or risperidone in a win-shift, 12-arm radial arm maze task (Terry et al., 2007a). Thus, antipsychotic effects in memory-related tasks in rodents appear to depend on the particular antipsychotic assessed and further, such behavioral effects appear to be time-dependent and task-specific. Such results also suggest that antipsychotics may differentially affect specific domains of cognition over time.

Differential effects in behavioral tasks that measure spatial learning are especially intriguing considering that radial arm maze and water maze rely on different motivational reinforcers as well as different levels of motor activity. This suggests that effects of FGAs (and possibly SGAs) on memory-task performance may not be strictly memory related per se. The negative effects of FGAs on motor activity are well established as are the anxiogenic effects of haloperidol, both in humans and animals (Ballard et al., 2007) In addition, on psychomotor driving tests, patients receiving typical antipsychotics scored worse than the general population as well as patients receiving atypical drugs especially on measures of attention, divided attention, and stress tolerance (Brunnauer et al., 2004). In a similar study, schizophrenics treated with risperidone or haloperidol both scored lower on the same psychomotor driving tests, but haloperidol-treated patients showed greater impairments than those using risperidone (Soyka et al., 2005). According to attentional control theory, anxiety disturbs attentional

processes and may result in decreased accuracy in testing and decreased efficiency of accomplishing a task (Eysenck et al., 2007). Accordingly, it is of interest to further investigate the effects of chronic antipsychotic treatment on certain behaviors that might influence performance of memory-related tasks (e.g., anxiety, psychomotor activity).

The purpose of the work described here was to further examine differential effects of chronic treatment by one representative first generation antipsychotic, haloperidol, and one second generation antipsychotic, risperidone, on acquisition and recall in water maze and radial arm maze procedures. The radial arm maze task was expanded to include a delayed nonmatch-to-position (DNMTP) version of the test as a more stringent (i.e., delay-dependent) task of working and short-term memory. The water maze procedure was designed to incorporate a longer training period allowing animals to reach an asymptotic level of performance. This extent of training provided appropriate conditioning for the addition of an extinction assessment using multiple probe trials. Extinction occurs when the relations among stimuli recognized during acquisition are no longer valid and the previously established responses are suppressed or more simply put, when experience does not match expectation (delta rule) (Widrow and Hoff, 1960; reviewed by Schmajuk and DiCarlo, 1992). Accordingly, preferences for a spatial location decrease in the water maze as the animal learns that the cues no longer predict the location of the hidden platform (Lattal and Abel, 2001). Extinction in this context is considered a type of cognitive flexibility, a form of fluid intelligence that encompasses the ability to inhibit strong response preferences in order to explore alternative solution paths (Beversdorf et al., 1999). Finally, in the water maze (specifically) we analyzed several nonmnemonic factors (e.g., anxiety and motor effects) that could influence task

performance (thigmotaxis, swim speeds, etc.).

METHODS

Animal Care

All procedures employed during this study were reviewed and approved by the Medical College of Georgia Institutional Animal Care and Use Committee and are consistent with AAALAC guidelines. Measures were taken to minimize pain or discomfort in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Significant efforts were also made to minimize the total number of animals used while maintaining statistically valid group numbers.

Male albino Wistar rats (Harlan Sprague-Dawley, Inc.) approximately two months old were housed singly in a temperature controlled room (25^{O}C), maintained on a reversed 12-hour light/dark cycle with free access to water. Food (Teklad Rodent Diet 8604 pellets, Harlan, Madison, WI) was given ad libitum except during testing for radial arm maze when it was restricted to maintain a target weight of approximately 325 grams. All animals were handled for four days prior to behavioral testing for two to five minutes during each handling session.

Drug Delivery

Oral antipsychotic dosing was based on several factors: 1) previous rodent studies in our laboratory in which time dependent behavioral and neurochemical effects were detected; 2) plasma drug levels were achieved that approximated those often

associated with antipsychotic effects in humans (Terry et al., 2002; Terry et al., 2003; Terry et al., 2005); 3) the doses selected (see below) were expected to achieve comparable and (therapeutically) relevant D_2 receptor occupancy values in vivo (i.e. in the range 65–80%, see (Kapur et al., 2003) based on the recent work of (Barth et al., 2006). Rats were thus treated with 1.0 or 2.0 mg/kg/day HAL (Sigma-Aldrich, St. Louis, MO) or 1.25 or 2.5 mg/kg/day risperidone (Pfizer, Inc., New York, NY) orally for a period of 33 (water maze) or 90 (radial arm maze) days. The antipsychotics were dissolved in 0.1 M acetic acid and subsequently diluted (1:100) with ultrapure water for daily drug administration in drinking water. Drug dosing was based on the average daily fluid consumption and the weight of the animals. Stability of the haloperidol and risperidone as concentrated solutions and when diluted in rodent drinking water was established in previous work by this lab (Terry et al., 2007b). All behavioral testing was begun on day 15 of drug exposure.

Preparation of Standard Solutions

Stock solutions of haloperidol and risperidone were prepared in 0.1 M acetic acid at concentrations of 5.0 and 6.25 mg/ml, respectively and kept in glass bottles in a refrigerator at 4 °C for up to 4 weeks. Dilutions of the concentrated solutions in tap water or deionized water (final concentrations of 20 µg/ml and 22.5 µg/ml for haloperidol and risperidone, respectively) were also prepared and transferred into standard rodent drinking water bottles with rubber stoppers and then stored for up to 96 h at room temperature.

Behavioral Testing

Radial Arm Maze

Testing was conducted in Med-Associates (MED-RAM-1R) computer-automated, 8-Arm Radial Arm Mazes consisting of a central octagonal hub (arena) with automatic guillotine doors connected to aluminum arms radiating distally (45.7 cm long). IR-photo beam sensors are positioned at the entrance to each runway, and a food pellet receptacle and head entry detector is positioned at the end of each runway. The maze is positioned approximately 90 cm above the floor in a testing room with a number of extra-maze cues (composed of large geometrical shapes). This computer-automated approach (used currently in our laboratory) is a modification of a method previously published (see Terry et al., 2001).

Habituation Phase. Test subjects were given two 15-minute free exploration (habituation) sessions prior to the Monday in which the win-shift portion of testing was conducted. This was done so that the animals became acquainted with the radial arm maze apparatus, as well as the handling procedures associated with it. Reinforcement food pellets were scattered randomly around the entire maze area during this session.

Acquisition (Win-Shift Training). After the habituation phase, subjects were trained in a win-shift procedure. A trial began when the experimenter placed the test subject into the central octagonal arena. After a 60 sec delay, all guillotine doors raised allowing access to all of the eight arms. When the animal broke a photo-beam in the pellet receptacle at the end of each runway a reward pellet was delivered once. When the rat moved back into the central arena all doors closed for 5 seconds and then reopened. All reentries into an arm that had previously delivered a reward were scored as working memory

errors. All animals were trained in win-shift (maze eight) for a minimum of 10 days. At the end of 10 days, animals advanced to the delayed nonmatch-to-position version (DNMTP or forced four-free eight) if criterion was met. Criterion of win-shift was completion of the task within the time limit (15 minutes) on four consecutive days with zero, one, or two errors.

Delayed Non-match to Position (DNMTP)- Testing began with an information (forced 4) session in which four of the eight arms were blocked. This session ended when all four arms were visited or when the trial timed out (15 min.). The animal remained in the testing room for the delay period. In the "free 8" (retention) test session, all eight arms were accessible, however, food reinforcement occurred only at the ends of the arms not visited in the previous information session. The test session continued until all four of the previously blocked arms were visited, or until 15 min. elapsed. The number of arm entries was recorded, along with two types of errors: reference-memory and working-memory errors. Following the second (test) session in each trial block, the animal is returned to its home cage in the housing facility, until the next day's information session. Animals were trained for a minimum of 10 days at a 15-minute delay between the forced four and the free eight sessions. When a criterion was met of four consecutive days at zero or one errors during the free eight sessions, animals advanced to longer delays of one, three, six, and 24 hours. Each longer delay was randomly presented twice.

Water maze

Water maze experiments were performed in a circular pool (180 cm in diameter, 76 cm in height) made of black plastic. The pool was filled to a depth of 35 cm with water

(maintained at 25.0 ± 1.0°C). The pool was located in a large room with a number of extra maze visual cues, including geometric images (e.g., squares, triangles, and circles) hung on the wall, ambient lighting of approximately 25 to 30 lox (lumens per square meter), and black curtains, used to hide the experimenter (visually) and the resting test subjects. Swimming activity of each rat was monitored via a television camera mounted overhead, which relayed information, including latency to find the platform, total distance traveled, time, and distance spent in each quadrant, to a video tracking system (Nodes, Leesburg, VA, USA).

Hidden platform task. For these experiments, an invisible (black) 10- x 10-cm square platform was submerged approximately 1.0 cm below the surface of the water and placed in the center of the northeast quadrant (which remained constant throughout hidden platform training). Each rat was given two trials per day for fourteen consecutive days to locate and climb onto the hidden platform. A trial was initiated by placing the rat in the water directly facing the pool wall (i.e., nose approximately two cm from the wall) in one of the four quadrants. The daily order of entry into individual quadrants was pseudorandom zed such that all four quadrants were used once every two training days. For each trial, the rat was allowed to swim a maximum of 90 s, to find the platform. When successful, the rat was allowed a 30-s rest period on the platform. If unsuccessful within the allotted time period, the rat was given a score of 90 s and then physically placed on the platform and also allowed the 30-s rest period. In either case, the rat was given the next trial after an additional 30 s rest period (i.e., intertribal interval 1.0 min).

Probe trials (transfer tests). Twenty-four hours following the last hidden platform trial, a probe trial lasting 60 s was conducted in which the platform was removed from the pool

to measure spatial bias for the previous platform location. This was accomplished by measuring the percentage of time spent in the previous target quadrant and the number of crossings over the previous platform location, and it provided a second estimate of the strength and accuracy of the memory of the previous platform location.

Extinction trials. In contrast with previous water maze studies, where we focused primarily on acquisition (hidden platform testing) and retention (probe trials), subjects were trained in the hidden platform tests to an asymptotic level of performance (defined as a latency to find the hidden platform of less than 20 sec for 4 consecutive trials). We have found in previous studies that 14 days (2 trials per day) is sufficient for antipsychotic treated rats (even those administered FGAs such as haloperidol) to reach this asymptotic level. At that point (i.e., on the following day after the last hidden platform test) we conducted four consecutive probe trials, 60 s in length separated by 60 s inter-trial intervals, to assess the subject's ability to decrease (i.e., extinguish) its spatial bias for the previous platform location. The animal entered the quadrant opposite the (previous) platform quadrant for the first trial, the platform quadrant for the second, a quadrant adjacent to the platform quadrant for the third, and the remaining quadrant for the fourth trial.

Visible platform task. Twenty-four hours after probe trials, a visible platform test was performed to ensure that the study subjects were visually capable of performing the task and that they demonstrated normal search/escape behaviors. To accomplish this task, a highly visible (white) cover fitted with a small white flag was attached to the platform (dimensions with cover attached 12 x 12 cm), which raised the surface approximately 1.0 cm above the surface of the water. Each rat was gently lowered into the water in the

quadrant diametrically opposite to the platform quadrant and given one or more trials with a 90-s time limit to locate and climb on to the platform. If unsuccessful after 90 s, it was physically placed on the platform for 30 s and then given a new trial. Once a rat was successful on its own accord, it was then given a series of four additional trials (with a 1.0-min intertrial interval) and the latency (in seconds) to locate the platform was recorded. The platform was moved on each trial to a different quadrant (the subject was always entered from the opposite quadrant) until the test was conducted once in all four quadrants.

Locomotor Activity and the Light/Dark Preference Test

To assess the effects of haloperidol on general locomotor activity as well as anxiety levels, a light/dark preference test (also referred to as light/dark exploration or emergence neophobia test) was conducted on day 12 of drug dosing (approximately 1 h after water maze testing; see below). This test is one of the most commonly used rodent models of anxiety (Holmes et al., 2001). Med Associates Inc. (St. Albans, VT) rat open field activity monitors (43.2 x 43.2 cm) were used for these experiments. They were fitted with dark box inserts (which are opaque to visible light) to cover half the open field area, thus separating the apparatus into two zones of equal area (i.e., a brightly lit zone and a darkened zone). Desk lamps located above the activity monitors were used to provide an illumination level of approximately 1000 lux (lumen/m^2) in the brightly lit zone, whereas the illumination level in the darkened zone was approximately 5 lux. The test was initiated by placing each subject into the lighted zone of the activity chamber. Activity (horizontal photobeam breaks) and the time spent in the light and dark zones of the apparatus was subsequently monitored and recorded continuously for 10 min.

Elevated Plus Maze

Elevated plus maze experiments were also conducted in the rats administered haloperidol 2.0 mg/kg (and vehicle-treated controls) as a second measure of anxiety levels (Pellow et al., 1985). Testing was conducted on day 22 of drug exposure. The elevated plus-maze was constructed from black Plexiglas and consisted of two open (50 × 10 cm) and two enclosed arms (50 × 10× 30 cm) extending from a central platform (10 × 10 cm) raised 107 cm above the floor. Ambient lighting was used in the room and light levels on the open and enclosed arms were similar. Each animal was placed on the central platform and allowed to explore the maze for 10 minutes. The time and distance traveled in the open arms were assessed using a video camera mounted on the ceiling above the apparatus and an automated tracking system LimeLight (Actimetrics, Wilmette, IL).

Statistical Analysis

The data were analyzed with SigmaStat version 2.03 software. For radial arm maze and watermaze training and extinction data, a two-way ANOVA with repeated measures was used for all treatment comparisons with Student Newman Keuls test for post hoc analyses. For visible platform, data were analyzed using a one-way ANOVA with repeated measures. To analyze probe trials, a one-way ANOVA was used. Elevated plus maze and light dark data were compared by analysis with t-tests. If normality failed in these two behavioral measures data was analyzed instead by Mann-Whitney Rank Sum Test. Differences were considered significant if the $p \leq 0.05$.

RESULTS

Radial Arm Maze

Win-shift. Figure 3.1A illustrates the lack of effect of either risperidone or haloperidol (n=12-13 per treatment group) on acquisition in a win-shift task in an automated, eight-arm radial arm maze. No significant difference was observed in the number of trials to criterion among groups ($F_{(4,62)}$=0.523, p=0.719). There was neither an overall treatment effect on errors committed by the subjects ($F_{(4,58)}$=1.290, p=0.285) nor a treatment by day effect ($F_{(10,692)}$=1.078, p=0.346). There was a significant effect of the day of testing ($F_{(10,692)}$=35.159, p<0.001) indicating that the test subjects clearly improved as they completed more trials.

Delayed Nonmatch-to-position. The results of the DNMTP training trials at 15-minute delays are presented in ure 3.1B. There were no significant differences detected in the trials to criterion ($F_{(4,60)}$=0.418, p=0.795), number of errors committed ($F_{(9,629)}$=0.933, p=0. 496), and there was no significant treatment by day effect on errors made across groups ($F_{(36,629)}$=1.144, p=0.264). With the introduction of longer delays (Figure 3.1C), increased length of delay paralleled increases in the number of errors across the treatment groups ($F_{(3,224)}$=32.528, p<0.001). There was no significant treatment effect on errors ($F_{(4,53)}$=1.626, p=0.181). There was also no significant treatment by delay interaction ($F_{(12,168)}$=0.819, p=0.631) however, further post hoc analyses revealed a trend (p<0.1) in which 2.5 risperidone animals had more errors than vehicles at the six hour delay.

Morris Water Maze

Hidden Platform Training. Figure 3.2 illustrates the results of experiments in which the FGA haloperidol (1.0. and 2.0 mg/kg/day) and the SGA risperidone (1.25 and 2.5 mg/kg/day) were evaluated for their ability to impair spatial learning in a water maze (n=15-21 per treatment group). The distance each experimental group traveled to locate the hidden platform over 14 consecutive days of testing is depicted in figure 3.2A. There was a highly significant treatment effect ($F_{(4,82)}$=4.752, p=0.002), a significant day effect ($F_{(13,52)}$=68.821, p<0.001), without a significant treatment x day interaction ($F_{(52,1066)}$=0.834, p=0.794). Post hoc analyses revealed that animals treated with haloperidol 2.0 swam significantly longer distances on days 9, 10, and 12 and haloperidal 1.0-treated animals traveled farther on days 9 and 10. The latency (number of seconds) of each experimental group to locate the hidden platform over days of testing is depicted in figure 3.2C. There was a highly significant treatment effect ($F_{(4, 82)}$=7.128, p<0.001), a significant day effect ($F_{(13, 1066)}$=63.986, p<0.001), without a significant treatment x day interaction ($F_{(52,1066)}$=0.926, p=0.625). Post hoc analyses indicated that the performance of controls (vehicle) was superior (p < 0.05) to performance of the animals administered haloperidol 2.0 on a majority of testing days. Treatment ($F_{(4,82)}$=4.596, p=0.002) and day ($F_{(13,52)}$=4.564, p<0.001) affected speed (fig. 3.3A), as well, but still there was no treatment by day effect ($F_{(52,1066)}$=0.921), p=0.633). According to post hoc analyses, vehicle-treated animals swam somewhat faster (p<0.05) than animals treated with either dose of haloperidol on days several days of testing. In a measurement of thigmotaxis (fig. 3.3B), there was a significant treatment effect ($F_{(4,82)}$=6.407, p<0.001) and day effect ($F_{(13,52)}$=79.174, p<0.001) while the

treatment by day effect was not significant ($F_{(52,1066)}$=0.806, p=0.836). Through post hoc analyses, we were able to discern that animals receiving HAL 2.0 mg/kg consistently showed thigmotaxic tendencies compared to vehicles across all days of training. Despite, differences in swim speed, swim distance, latency, and thigmotaxis, there was no significant difference in latency to the platform during visible platform trials ($F_{(4,82)}$=2.529, p=0.047; fig. 3.1B).

Water Maze Probe Trials and Extinction Test. Probe trial results for all animals are shown in figure 3.4A. There was a significant treatment effect on the number of platform crossings ($F_{(4,86)}$=2.529, p=0.047). Post hoc analyses revealed a trend (p<0.1) in which haloperidol 2.0-treated animals crossed the former platform position fewer times than vehicle-treated animals. Figure 3.4B illustrates the results of the extinction probe trials of the animals performing the fourteen-day training sessions and reaching the platform in less than 20 seconds for four consecutive trials. Treatment did not significantly affect the number of platform crossings ($F_{(4,39)}$=1.236). There was a significant difference in number of platform crossings across trials ($F_{(3,12)}$=12.755, p<0.001). Specifically, all treatment groups showed extinction (ie, crossed the previous platform area fewer times in trial 4 than in trials 1 and 2). There was no significant treatment by trial effect ($F_{(117,175)}$=1.236).

Light Dark Preference

The results of the light dark preference test are shown in figure 3.5A. As indicated, haloperidol-treated animals traveled similar total distances in the apparatus compared to vehicle-treated subjects ($t_{(17)}$=1.543, p=0.141) and there was no significant difference

in the time spent in the brightly lit area ($t_{(17)}$=1.482, p=0.157). However, the distance traveled in the brightly lit area was significantly lower in the haloperidol-treated animals compared to controls ($t_{(17)}$=2.248, p=0.027).

Elevated Plus Maze

In the elevated plus maze (fig. 3.5B), haloperidol treated subjects traveled shorter total distances ($t_{(18)}$=8.208, p<0.001), shorter distances in open arms (HAL median quartile=12.250 and VEH median =194.90, p=0.045), and spent significantly less time in the open arms in comparison to animals receiving vehicle ($t_{(18)}$=2.142, p=0.046).

DISCUSSION

The results of the experiments conducted in this study can be summarized as follows: 1) In radial arm maze experiments neither haloperidol nor risperidone affected win-shift acquisition although DNMTP performance was modestly impaired at the longer delays by risperidone; 2) haloperidol, but not risperidone, impaired water maze hidden platform acquisition as well as probe trial performance; 3) risperidone was without significant effect in the subsequent extinction trials and due to impairments in the first probe trial, it was not possible to fully assess the effects of haloperidol on extinction; 4) the level of thigmotaxis was elevated and swim speeds were somewhat reduced in the haloperidol-treated animals in the water maze possibly indicative of psychomotor impairments and elevated levels of anxiety; 5) the possible influence of elevated levels of anxiety in haloperidol-treated animals on the performance of memory-related tasks was further supported by subsequent experiments using the light-dark box and elevated plus maze tests.

Since haloperidol-treated animals performed similarly to the other groups in radial arm maze, one might surmise that the impairment seen in the watermaze task was motor related. Although alterations in motor skills may have influenced the behavioral results, haloperidol-treated rats performed similarly in visible platform trials in the water maze and showed no differences in total distance traveled in light/dark preference test. In addition, even though haloperidol-treated animals swam at slightly slower speeds in the water maze, they swam longer distances to find the hidden platform which is clearly indicative of learning impairment. Collectively, this finding would argue against the idea that gross motor impairment could explain the water maze performance deficits. Similar performance in extinction trials would appear to indicate that haloperidol, risperidone, and vehicle treated animals are equally capable of cognitive flexibility. However, fewer haloperidol 2.0-treated animals progressed to extinction trials than vehicle animals since they did not meet the set learning criteria in the hidden platform test**.** In addition, haloperidol-treated animals crossed the previous platform area less within the first two trials, and the variance was higher making conclusions from these experiments difficult

The disparate results observed in the water maze and radial arm mazes (i.e., both within and between antipsychotic groups) were intriguing. Although the water maze and radial arm maze are both tasks designed to test visuospatial learning and memory, the tasks are quite different (reviewed by Hodges, 1996). Other studies, (Nelson et al., 1997; Nunn et al., 1998; Pouzet et al., 1999), have found differences across spatial memory tasks as well. Key differences between water maze and radial arm maze are Morris water maze is a less complex task based on spontaneous exploration of an open field type arena with aversive escape compared to the appetitive driven complex choice

sequence of radial arm maze. The water maze task offers fewer visual cues as run by our laboratory and virtually eliminates olfactory cues. This limits the microchoices (Brown, 1992; Brown et al., 1993) afforded by more and more evenly distributed extra- and intra-maze cues present in radial arm maze testing. In our laboratory, this is especially true since the water maze is conducted surrounded by a thick black curtain except for two opposing openings allowing a view of two sets of visual cues located on walls four to six feet from the pool location. In contrast, the walls of the automated radial arm mazes are clear plexiglass allowing views of visual cues located throughout the room including maze hardware located adjacent to maze arms. Ethologically, radial arm maze relies on more natural foraging behaviors of rats and allows the use of olfaction, vibrissae sensation, along with the visual cues and navigation.

Search strategy and mechanism are yet other important factors in both water maze and radial arm maze. Thigmotaxis may indicate heightened anxiety when practiced by a rodent in an open field task (Treit and Fundytus, 1988). Typically, normal animals exhibit thigmotaxis or a preference for the outer rim of an area, when initially exposed to a new arena. This allows the individual to search the perimeter for an escape access. As exposure continues, this search strategy is abandoned for one that more fully explores the arena including center crosses. In humans participating in maze simulations, thigmotaxis indicates impairment only if the switch to a more effective search strategy never occurs even after arena habituation (Graziano et al., 2003; Kallai et al., 2005). Studies in people also show thigmotaxis is a sign of fear more than anxiety. Devan (1999) showed in animals caudate putamen lesions brought about thigmotaxis in water maze but postulated it was still related to anxiety because of

cortical and limbic connections. Interestingly, increased anxiety is listed as an adverse effect listed on package inserts of haloperidol and has been shown to decrease tolerance to stress in people exposed to psychomotor driving tests (Soyka et al., 2005). Anxiety may disturb attentional processes and information integration. In addition, neuroleptic induced akathisia (a movement disorder characterized by restlessness, agitation, and anxiety) in patients was recently associated with cognitive dysfunction (Kim and Byun, 2007).

In conclusion, the results of these studies indicate that subchronic exposure to therapeutic doses of haloperidol (but not risperidone) leads to impairments in the performance of a spatial reference learning procedure. These deficits may in part be due to haloperidol-related psychomotor impairments and elevated levels of anxiety. Further, risperidone may impair spatial working and short-term memory as the demands of the task increase.

AKNOWLEDGEMENTS

This work was supported in part by the American Foundation for Pharmaceutical Education's Pre-doctoral Fellowship program and by the National Institute of Mental Health (MH 066233 to AVT).

REFERENCES

Ballard M.E., Basso A.M., Gallagher K.B., Browman K.E., Fox G.B., Drescher K.U., Gross G., Decker M.W., Rueter L.E., Zhang M. 2007. The drug-induced helplessness test: an animal assay for assessing behavioral despair in response to neuroleptic treatment. *Psychopharmacology* (Berl) *190*, 1-11.

Barth V.N., Chernet E., Martin L.J., Need A.B., Rash K.S., Morin M., Phebus L.A. 2006. Comparison of rat dopamine D2 receptor occupancy for a series of antipsychotic drugs measured using radiolabeled or nonlabeled raclopride tracer. *Life Sci 78*, 3007-3012.

Beversdorf D.Q., Hughes J.D., Steinberg B.A., Lewis L.D., Heilman K.M. 1999. Noradrenergic modulation of cognitive flexibility in problem solving. *Neuroreport 10*, 2763-2767.

Brown M.F. 1992. Does a cognitive map guide choices in the radial-arm maze? *J Exp Psychol Anim Behav Process 18*, 56-66.

Brown M.F., Rish P.A., VonCulin J.E., Edberg J.A. 1993. Spatial guidance of choice behavior in the radial-arm maze. *J Exp Psychol Anim Behav Process 19*, 195-214.

Brunnauer A., Laux G., Geiger E., Moller H.J. 2004. The impact of antipsychotics on psychomotor performance with regards to car driving skills. *J Clin Psychopharmacol 24*, 155-160.

Crespo-Facorro B., Perez-Iglesias R., Ramirez-Bonilla M., Martinez-Garcia O., Llorca J., Luis Vazquez-Barquero J. 2006. A practical clinical trial comparing haloperidol, risperidone, and olanzapine for the acute treatment of first-episode nonaffective psychosis. *J Clin Psychiatry 67*, 1511-1521.

Devan B.D., McDonald R.J., White N.M. 1999. Effects of medial and lateral caudate-putamen lesions on place- and cue-guided behaviors in the water maze: relation to thigmotaxis. *Behav Brain Res 100*. 5-14.

Eysenck M.W, Derakshan N., Santos R., Calvo M.G. 2007. Anxiety and cognitive performance: attentional control theory. *Emotion 7*, 336-353.

Graziano A., Petrosini L., Bartoletti A. 2003. Automatic recognition of explorative strategies in the Morris water maze. *J Neurosci Methods 130*, 33-44.

Green M.F. 1996. What are the functional consequences of neurocognitive deficits in schizophrenia? *Am J Psychiatry 153*, 321-330.

Green M.F. 2006. Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. *J Clin Psychiatry 67 Suppl 9*, 3-8; discussion 36-42.

Green M.F., Marder S.R., Glynn S.M., McGurk S.R., Wirshing W.C., Wirshing D.A., Liberman R.P.., Mintz J. 2002. The neurocognitive effects of low-dose haloperidol: a two-year comparison with risperidone*. Biol Psychiatry 51*, 972-978.

Hodges H. 1996. Maze procedures: the radial-arm and water maze compared. *Brain Res Cogn Brain Res 3*,167-181.

Holmes A., Iles J.P., Mayell S.J., Rodgers R.J. 2001. Prior test experience compromises the anxiolytic efficacy of chlordiazepoxide in the mouse light/dark exploration test. *Behav Brain Res 122*,159-167.

Kallai J., Makany T., Karadi K., Jacobs W.J. 2005 Spatial orientation strategies in Morris-type virtual water task for humans. *Behav Brain Res 159,*187-196.

Kapur S., VanderSpek S.C., Brownlee B.A., Nobrega J.N. 2003. Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: A suggested solution based on in vivo occupancy. *J Pharmacol Exper Ther 305*, pp 625-631.

Keefe R.S., Bilder R.M., Davis S.M., Harvey P.D., Palmer B.W., Gold J.M., Meltzer H.Y., Green M.F., Capuano G., Stroup T.S., McEvoy J.P., Swartz M.S., Rosenheck R.A., Perkins D.O., Davis C.E., Hsiao J.K., Lieberman J.A. 2007. Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE Trial. *Arch Gen Psychiatry ,* 633-647.

Keefe R.S.E., Seidman L.J., Christensen B.K., Hamer R.M., Sharma T., Sitskoorn M.M., Lewine R.R.J., Yurgelun-Todd D.A., Gur R.C., Tohen M., Tollefson G.D., Sanger T.M., Lieberman J.A., Group H.R. 2004. Comparative effect of atypical and conventional antipsychotic drugs on neurocognition in first-episode psychosis: A randomized, double-blind trial of olanzapine versus low doses of haloperidol. *Am J Psychiatry 161*, 985-995.

Kessler R.M., Ansari M.S., Riccardi P., Li R., Jayathilake K., Dawant B., Meltzer H.Y. 2005. Occupancy of striatal and extrastriatal dopamine D2//D3 receptors by olanzapine and haloperidol. *Neuropsychopharmacology 30*, 2283-2289.

Kim J.H., Byun H.J. 2007. Association of subjective cognitive dysfunction with akathisia in patients receiving stable doses of risperidone or haloperidol. *J Clin Pharm Ther 32*, 461-467.

Kurtz M.M. 2006. Symptoms versus neurocognitive skills as correlates of everyday functioning in severe mental illness. *Expert Rev Neurother 6*, 47-56.

Kurtz M.M., Moberg P.J., Ragland J.D., Gur R.C., Gur R.E. 2005. Symptoms versus neurocognitive test performance as predictors of psychosocial status in schizophrenia: A 1- and 4-year prospective study. *Schizophr Bull 31*,167-174.

Lattal K.M., Abel T. 2001. Different requirements for protein synthesis in acquisition and extinction of spatial preferences and context-evoked fear. *J Neurosci 21*, 5773-5780.

Nelson A., Sowinski P., Hodges H. 1997. Differential effects of global ischemia on delayed matching- and non-matching-to-position tasks in the water maze and Skinner box. *Neurobiol Learn Mem 67*, 228-247.

Nunn J.A., Gray J.A., Hodges H. 1998. Neurotoxic dorsal CA1 lesions versus 4 VO ischaemic lesions: behavioural comparisons. *Behav Neurol 11,* 217-226.
Pellow S., Chopin P., File S.E., Briley M. 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods 14,*149-167.

Pinkham A.E., Penn D.L. 2006. Neurocognitive and social cognitive predictors of interpersonal skill in schizophrenia. *Psychiatry Res 143*,167-178.

Polsky D., Doshi J.A., Bauer M.S., Glick H.A. 2006. Clinical trial-based cost-effectiveness analyses of antipsychotic use. *Am J Psychiatry 163*, 2047-2056.

Pouzet B., Welzl H., Gubler M.K., Broersen L., Veenman C.L., Feldon J., Rawlins J.N., Yee B.K. 1999. The effects of NMDA-induced retrohippocampal lesions on performance of four spatial memory tasks known to be sensitive to hippocampal damage in the rat. *Eur J Neurosci 11*,123-140.

Sanger T.M., Lieberman J.A., Tohen M., Grundy S., Beasley C., Jr., Tollefson G.D. 1999. Olanzapi ne versus haloperidol treatment in first-episode psychosis. *Am J Psychiatry 156*,79-87.

Schmajuk N.A., DiCarlo J.J. 1992). Stimulus configuration, classical conditioning, and hippocampal function. *Psychol Rev 99*, 268-305.

Schooler N., Rabinowitz J., Davidson M., Emsley R., Harvey P.D., Kopala L., McGorry P.D., Van Hove I., Eerdekens M., Swyzen W., De Smedt G., the Early Psychosis Global Working Group. 2005. Risperidone and haloperidol in first-episode psychosis: A long-term randomized trial. *Am J Psychiatry 162*, 947-953.

Seeman P. 2002. Atypical antipsychotics: mechanism of action. *Can J Psychiatry 47*, 27-38.

Shirzadi A.A., Ghaemi S.N. 2006. Side Effects of Atypical Antipsychotics: Extrapyramidal Symptoms and the Metabolic Syndrome. *Informa Healthcare 14*,152 - 164.

Soyka M., Winter C., Kagerer S., Brunnauer M., Laux G., Moller H.J. 2005. Effects of haloperidol and risperidone on psychomotor performance relevant to driving ability in schizophrenic patients compared to healthy controls. *J Psychiatr Res 39*, 101-108.

Terry A.V., Jr., Gearhart D.A., Mahadik S.P., Warsi S., Davis L.W., Waller J.L. 2005. Chronic exposure to typical or atypical antipsychotics in rodents: temporal effects on central alpha7 nicotinic acetylcholine receptors. *Neuroscience 136*, 519-529.

Terry A.V., Jr., Gearhart D.A., Warner S., Hohnadel E.J., Middlemore M.L., Zhang G., Bartlett M.G., Mahadik S.P. 2007a. Protracted effects of chronic oral haloperidol and risperidone on nerve growth factor, cholinergic neurons, and spatial reference learning in rats. *Neuroscience* Sept 14, Epub ahead of print.

Terry A.V., Jr., Gearhart D.A., Warner S.E., Zhang G., Bartlett M.G., Middlemore M.L., Beck W.D., Jr., Mahadik S.P., Waller J.L. 2007b. Oral haloperidol or risperidone treatment in rats: temporal effects on nerve growth factor receptors, cholinergic neurons, and memory performance. *Neuroscience 146*,1316-1332.

Terry A.V., Jr., Hernandez C.M., Buccafusco J.J. 2001. Dahl salt-sensitive and salt-resistant rats: examination of learning and memory performance, blood pressure, and the expression of central nicotinic acetylcholine receptors. *Neuroscience 103*, 351-363.

Terry A.V., Jr., Hill W.D., Parikh V., Evans D.R., Waller J.L., Mahadik S.P. 2002. Differential effects of chronic haloperidol and olanzapine exposure on brain cholinergic markers and spatial learning in rats. *Psychopharmacology (Berl) 164*, 360-368.

Terry A.V., Jr., Hill W.D., Parikh V., Waller J.L., Evans D.R., Mahadik S.P. 2003. Differential effects of haloperidol, risperidone, and clozapine exposure on cholinergic markers and spatial learning performance in rats. *Neuropsychopharmacology 28*, 300-309.

Treit D., Fundytus M. 1988. Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacol Biochem Behav 31*, 959-962.

Villalta-Gil V., Vilaplana M., Ochoa S., Haro J.M., Dolz M., Usall J., Cervilla J. 2006. Neurocognitive performance and negative symptoms: are they equal in explaining disability in schizophrenia outpatients? *Schizophr Res 87,* 246-253.

Widrow B., Hoff M. 1960. Adaptive switching circuits. In: 1960 WESCON Convention Record Part IV, pp 96-104 New York.

FIGURE LEGENDS

Fig 3.1. Effects of subchronic treatment with haloperidol or risperidone (compared with vehicle controls) on the performance of a water maze hidden platform procedure. Testing began on day 15 of drug exposure. **(A)** Acquisition curve of win-shift, each point represents the mean errors per trial during first 10 days of training (±S.E.M). Inset shows win-shift trials to criterion (±S.E.M). **(B)** Trials to criterion for RAM DNMTP training with 15 minute delays. **(C)** Mean errors committed per free-eight trial for each treatment group (±S.E.M) for DNMTP with 15 minute, 1 hour, 3 hour, and 6 hour delays between forced-four and free-eight sessions. #=1.0 Hal mg/kg/day animals significantly different from vehicle (P<0.05). *= 2.0 Hal mg/kg/day animals significantly different than vehicle controls (P<0.05). N=15-21 rats/group. VEH=vehicle HAL=haloperidol RIS=Risperidone

Fig 3.2. Effects of subchronic treatment with haloperidol or risperidone (compared with vehicle controls) on the performance of a water maze hidden platform procedure. Testing began on day 15 of drug exposure. **(A)** acquisition curve for distance swam, each point represents the mean swim distance to the hidden platform (±S.E.M) of two trials/day over 14 consecutive days of testing. **(B)** Mean latency to platform during visible platform trials. **(C)** acquisition curve for latency to reach hidden platform, each point represents the mean latency to the hidden platform (±S.E.M) of two trials/day over 14 consecutive days of testing. #=1.0 Hal mg/kg/day animals significantly different from vehicle (P<0.05). *= 2.0 Hal mg/kg/day animals significantly different than vehicle controls (P<0.05). N=15-21 rats/group. VEH=vehicle HAL=haloperidol RIS=Risperidone

Fig. 3.3. Effects of subchronic treatment with haloperidol or risperidone (compared with

vehicle controls) on the performance of a water maze hidden platform procedure. Testing began on day 15 of drug exposure. **(A)** Swim speed. Each point represents the mean swim speed (±S.E.M) of two trials/day over 14 consecutive days of testing. **(B)** Thigmotaxis. Each point represents the mean percent of time an animal spent in the outer periphery (20 cm annulus around outer rim of pool) (±S.E.M) of two trials/day over 14 consecutive days of testing. #=1.0 Hal mg/kg/day animals significantly different from vehicle (P<0.05). *= 2.0 Hal mg/kg/day animals significantly different than vehicle controls (P<0.05). N=15-21 rats/group. VEH=vehicle HAL=haloperidol RIS=Risperidone

Fig. 3.4. Effects of subchronic treatment with haloperidol or risperidone (compared with vehicle controls) on the performance of water maze probe trials and extinction trials. Probe trials and extinction trials were conducted on the 15th day of water maze testing and day 29 of drug exposure. **(A)** The mean number of crossings over the previous (10×10 cm) platform area (mean±S.E.M.) is depicted for all animals of each treatment group. **(B)** For extinction trials, the mean number of crossings over the previous (10×10 cm) platform area (mean±S.E.M.) for each of 4 consecutive trials (with a 1 min ITI) is depicted for animals of each treatment group that met criterion of 4 consecutive hidden platform trials with a latency of 20s or less. #=1.0 Hal mg/kg/day animals significantly different from vehicle (P<0.05). *= 2.0 Hal mg/kg/day animals significantly different than vehicle controls (P<0.05). N=15-21 rats/group. VEH=vehicle HAL=haloperidol RIS=Risperidone

Fig. 3.5. Effects of subchronic treatment with haloperidol compared with vehicle controls in (A) light-dark preference test and (B) the elevated plus maze. Light dark preference test (10 minutes in length) conducted on day 15 of exposure. Fear/anxiety-related

behavior (emergence neophobia) measured as the distance traveled in a brightly lit zone of the activity monitor. Elevated plus maze test (10 minutes in length) conducted on day 22 of exposure. Fear/anxiety-related behavior measured as length of time spent in center or open arms. Bars represent the mean±S.E.M. *N*=10 rats/group. * Significantly different (*P*<0.05) than vehicle controls; * significantly difference between the haloperidol and vehicle VEH=vehicle HAL=haloperidol

Fig 3.1

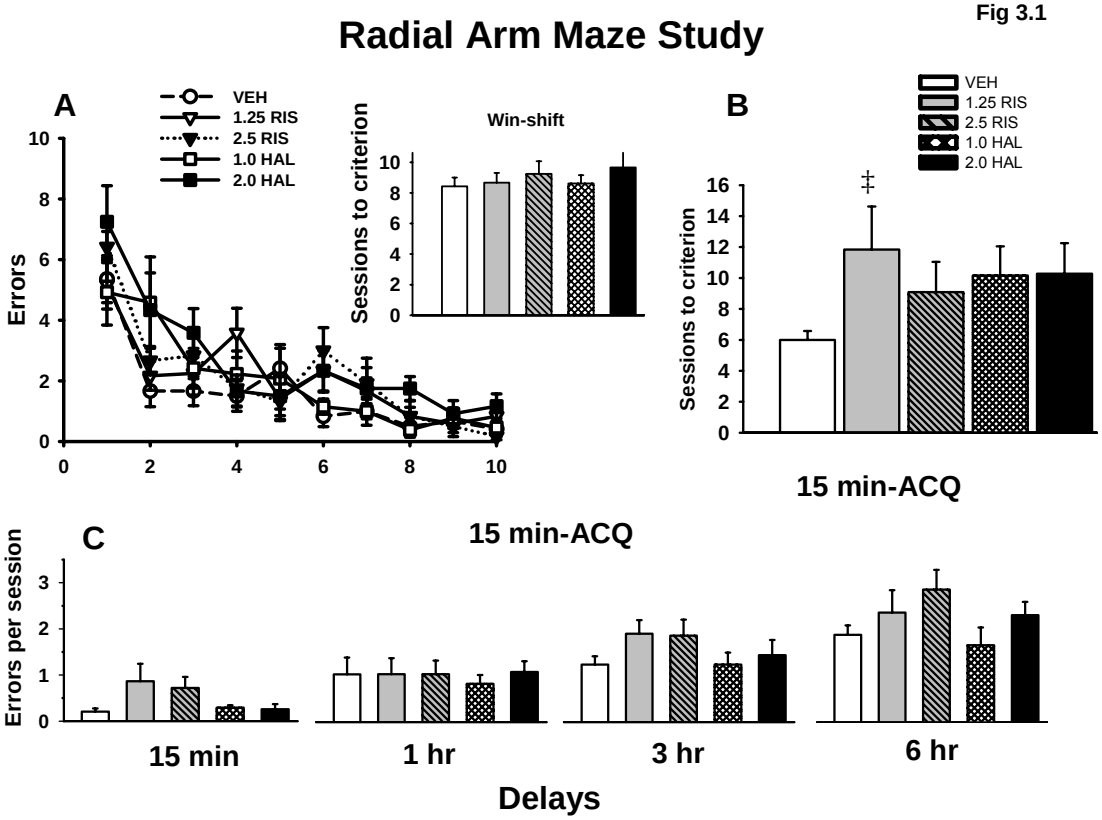

Radial Arm Maze Study
A
VEH
1.25 RIS
2.5 RIS
1.0 HAL
2.0 HAL
Errors
Win-shift
Sessions to criterion
B
VEH
1.25 RIS
2.5 RIS
1.0 HAL
2.0 HAL
‡
Sessions to criterion
15 min-ACQ
C
15 min-ACQ
Errors per session
15 min
1 hr
3 hr
6 hr
Delays

Figure 2

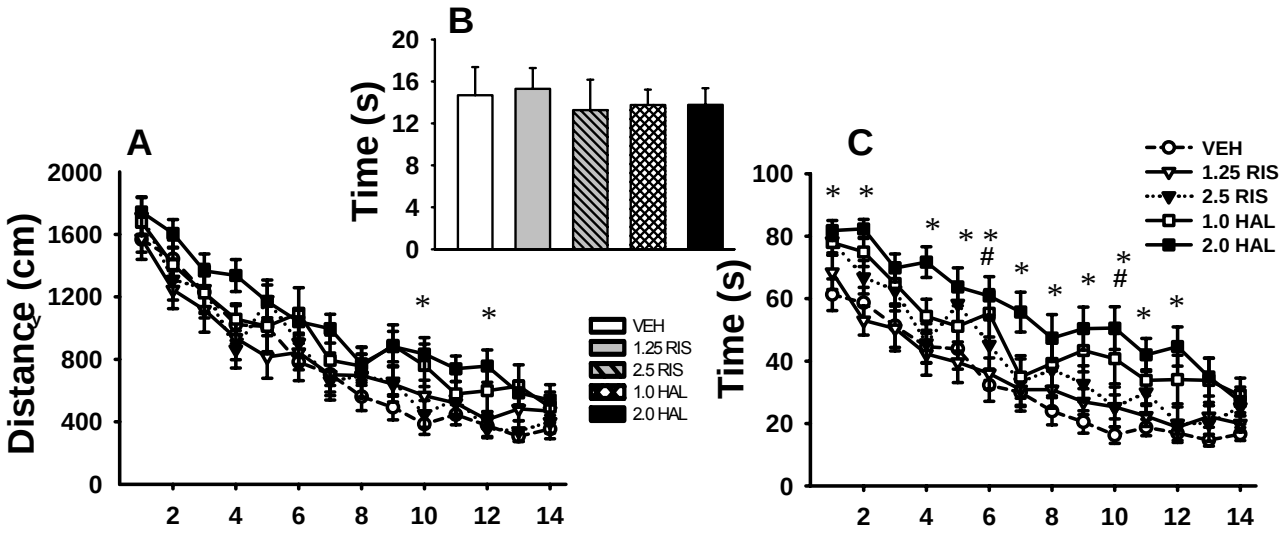

A
Distance (cm)
2000
1600
1200
800
400
0
2
4
6
8
10
12
14
B
Time (s)
20
16
12
8
4
0
VEH
1.25 RIS
2.5 RIS
1.0 HAL
2.0 HAL
C
Time (s)
100
80
60
40
20
0
VEH
1.25 RIS
2.5 RIS
1.0 HAL
2.0 HAL

Fig. 3.3

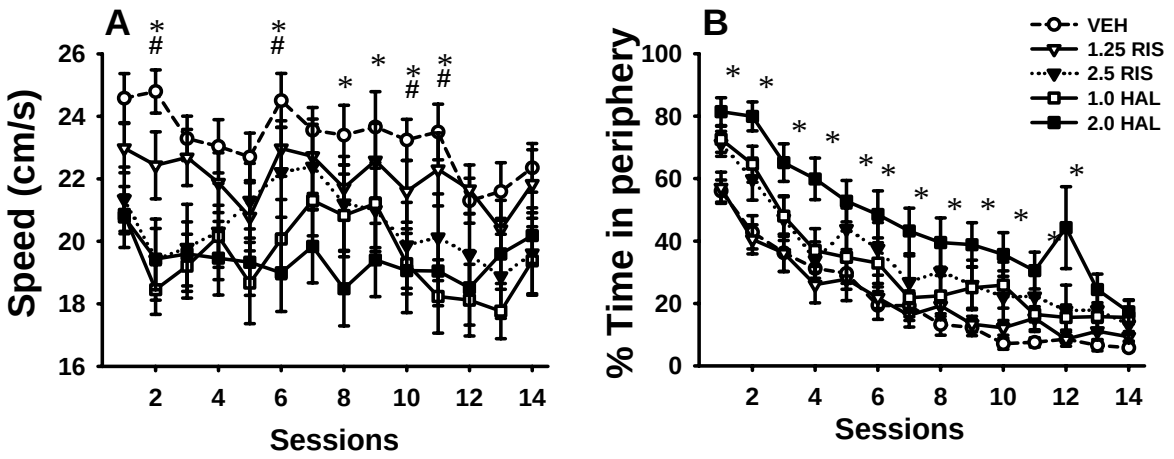

A
Speed (cm/s)
26
24
22
20
18
16
2
4
6
8
10
12
14
Sessions
B
% Time in periphery
100
80
60
40
20
0
2
4
6
8
10
12
14
Sessions
VEH
1.25 RIS
2.5 RIS
1.0 HAL
2.0 HAL

Fig. 3.4

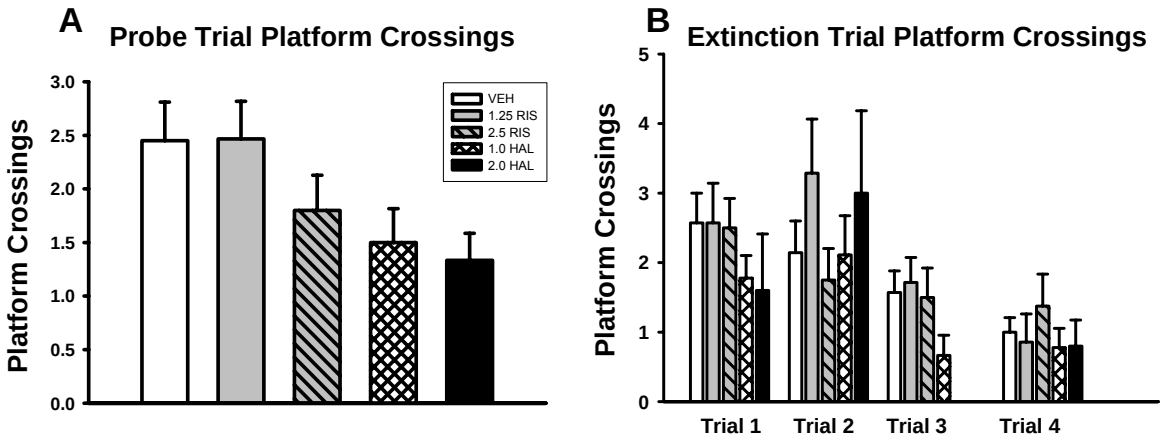
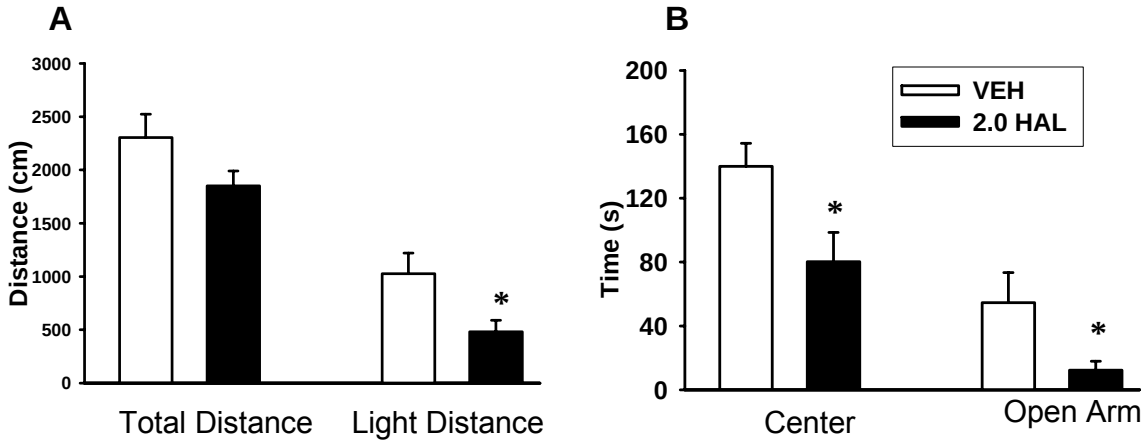

A
Probe Trial Platform Crossings
Platform Crossings
3.0
2.5
2.0
1.5
1.0
0.5
0.0
VEH
1.25 RIS
2.5 RIS
1.0 HAL
2.0 HAL
B
Extinction Trial Platform Crossings
Platform Crossings
5
4
3
2
1
0
Trial 1
Trial 2
Trial 3
Trial 4

Fig. 3.5


A
3000
2500
2000
1500
1000
500
0
Distance (cm)
*
Total Distance
Light Distance
B
200
160
120
80
40
0
Time (s)
VEH
2.0 HAL
*
*
Center
Open Arm

CHAPTER 4

CHRONIC HALOPERIDOL AND RISPERIDONE TREATMENT DISRUPT ACQUISTION IN A TEST OF SUSTAINED ATTENTION IN RATS WITHOUT ALTERATION OF VACHT AND A7NACHR LEVELS IN MEDIAL PREFRONTAL CORTEX

Hohnadel, E., J. Truan, and A.V. Terry, Jr. To be submitted to Neuropharmacology.

ABSTRACT

First and second generation antipsychotics (FGAs and SGAs respectively) have been demonstrated to effectively control the positive symptoms of schizophrenia. However, while SGAs may improve the negative symptoms; neither class of antipsychotic has been clearly associated with robust improvements of the debilitating cognitive deficits. This is important since the cognitive deficits (particularly in the domains of memory, executive function, and vigilance) appear to have the greatest impact on long-term functional outcome. Given the debilitating extrapyramidal symptoms associated with FGAs, the high costs of SGAs, combined with their recently identified adverse side effects (hyperlipidemia, diabetes, weight gain, etc.) selecting optimal therapies for patients is difficult. Animal studies (particularly involving chronic antipsychotic administration) may provide insight for developing optimal treatment strategies, especially when analogous behavioral tests to examine specific domains of cognition are available for humans and animals. Sustained attention and vigilance as well as other components of executive function (all disrupted in schizophrenia) can be measured with the continuous performance task/test (CPT) in humans and with the five-choice serial reaction time task (5-CSRTT) in rodents. The cholinergic neurotransmitter system is important in these cognitive processes and performance of these tasks. The purpose of this work was to determine in rats if long-term treatment (i.e., up to 320 days) with one representative FGA, haloperidol, or one representative SGA, risperidone, affected the acquisition of the 5-CSRTT and further, if either drug altered the levels of two cholinergic markers, vesicular acetylcholine transporter (VAChT) or the

alpha-7 nicotinic acetylcholine receptor (α7 nAChR), in the medial prefrontal cortex (i.e., a brain region critical to 5-CSRTT performance). Haloperidol severely impaired 5-CSRTT acquisition as indicated by the failure of subjects to meet specific performance criteria at the longer (i.e., less difficult) stimulus durations (SDs). While the risperidone-treated rats were capable of acquiring the task, fewer subjects were able to meet the performance criterion associated with each SD. General increases in response and reward latencies were observed across the study (i.e., in all of the antipsychotic-treated groups), leading to the conclusion that alterations in 5C-SRTT acquisition may in part be due to alterations in signal detection, psychomotor speed, and/or reward motivation Finally, neither haloperidol nor risperidone significantly affected VAChT or α7 nAChR levels in the medial prefrontal cortex in subsequent ELISA experiments. The results of these studies thus indicate that chronic exposure to therapeutic doses of haloperidol and risperidone (to a lesser degree) leads to impairments in the acquisition of a task of sustained attention, vigilance, and executive function. These deficits do not appear to be due to deficits in VAChT or the α7 nAChR in medial prefrontal cortex.

INTRODUCTION

While the positive and negative symptoms of schizophrenia have long been recognized as characteristic features, cognitive deficits (particularly in the domains of memory, executive function, and vigilance) appear to have the greatest impact on long-term functional outcome. (Green, 1996; Kurtz et al., 2005; Pinkham and Penn, 2006; Villalta-Gil et al., 2006). Current therapies for schizophrenia effectively control positive symptoms and second generation antipsychotics (SGAs also referred to as atypical antipsychotics) appear to ameliorate the negative symptoms. However, neither first generation antipsychotics (FGAs) nor SGAs robustly improve the debilitating cognitive deficits (Keefe et al., 2007). It is yet unclear which treatment is optimal for patients when considering this point along with high costs of SGAs, expenses related to hospitalization during relapse, and the risk of adverse effects (Davies et al., 2007; reviewed Rosenheck, 2007). Extrapyramidal symptoms are typically associated with FGAs, but SGAs at larger doses may also cause extrapryamidal symptoms (reviewed Correll et al., 2004) in addition to placing patients at increased risk for weight gain, hyperlipidemia, and diabetes mellitus in patients (reviewed Shirzadi and Ghaemi, 2006).

In order to determine the most efficacious therapy with the least adverse effects, animal studies involving chronic neuroleptic administration provide insight along with clinical studies, especially when analogous tests exist to examine components of cognition in both humans and rodents. In MATRICS (Measurement And Treatment Research to Improve Cognition in Schizophrenia), a team of researchers and clinicians recognized

eight separable cognitive factors most commonly affected in schizophrenia: Speed of Processing, Attention/Vigilance, Working Memory, Verbal Learning and Memory, Visual Learning and Memory, Reasoning and Problem Solving, Verbal Comprehension, and Social Cognition (Nuechterlein et al., 2004). Sustained attention and vigilance as well as some parameters of executive function can be measured with the continuous performance task/test (CPT) in humans (Beck et al., 1956; Mackworth and Taylor, 1963) and with the five-choice serial reaction time task (5-CSRTT) in rodents (review by Robbins, 2002). Executive function contributes to reasoning and problem solving and is defined as cognitive control for optimizing planning, scheduling complex behavior, attentional selection and resistance to interference, monitoring, behavioral inhibition, task switching, planning, and decision making (Dalley et al., 2004a).

The rodent analogue to the continuous performance task is the five-choice serial reaction time task (5-CSRTT). This task measures sustained attention, but other behaviors have been associated with test parameters. Errors of omission constitute inattention (Risbrough et al., 2002). Longer latencies to respond or to collect reward pellets display lack of motivation (Robbins, 2002). Because they show a failure to inhibit inappropriate responses, premature and perseverative nose pokes are linked to executive behavior. Premature responses have been compared to impulsive behavior in people (Carli et al., 1983; Chudasama et al., 2003; Dalley et al., 2004b) while perseverative responses are considered equivalent to compulsive behavior (Robbins, 2002). Through lesional studies and through imaging technology using metabolic monitoring, brain areas connected to each behavior have been identified in rodents

(reviewed by Dalley et al., 2004b; Robbins, 2002). Attentional selectivity is a function of the pregenual area and anterior cingular cortex (Passetti et al., 2002). However, impulsive activity seems to be generated from the infralimbic cortex (Chudasama et al., 2003) and perseveration reactions are a product of the prelimbic cortex (Chudasama and Muir, 2001). The pregenual area, anterior cingulated cortex, infralimbic cortex, and prelimbic cortex are sometimes considered together as medial prefrontal cortex which has been considered analogous to dorsal lateral prefrontal cortex in humans (reviewed (Uylings et al., 2003), the area involved in CPT performance and some components of executive function.

The importance of the cholinergic system in attention, learning, and memory is widely published. In tests of sustained attention, acetylcholine is an essential part of the neuronal chemistry influencing performance in both CPT and 5-CSRTT. In rats, 5CSRTT testing mediates increased release of Ach in medial prefrontal cortex but the amount appears to be independent of the difficulty (i.e. stimulus durations or intertribal intervals) of the session (Passetti et al., 2000). Furthermore, impairments imposed by excitotoxic lesions of basal forebrain are partially reversed by physotigmine or nicotine (Muir et al., 1995). The greatest effects of nucleus basalis lesions are on ChAT activity in the prefrontal cortex which correlates positively with decreases in accuracy on 5CSRTT. Scopolamine, a muscarinic receptor antagonist, and mecamylamine, a nicotinic receptor antagonist, have also been shown to impair 5CSRTT performance (Jones et al., 1995). Meanwhile, in humans it is well documented that nicotine improves sustained attention and CPT performance (reviewed Rezvani and Levin, 2004).

Some evidence suggests that cholinergic alterations may be present in schizophrenia patients. For example, reductions in choline acetyltransferase (reviewed Powchik et al., 1998), low affinity (α_7) nicotinic acetylcholine receptors (nAChRs), high affinity ($\alpha_4\beta_2$) nAChRs (reviewed Friedman, 2004), and M_1/M_4 muscarinic acetylcholine receptors (mAChRs) (Crook et al., 2000; Crook et al., 2001; Dean et al., 1996) have been reported. 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 (Terry et al., 2007a), the vesicular acetylcholine transporter, and nicotinic (alpha(7)) (Terry and Gearhart, 2007) and muscarinic (M(2)) acetylcholine receptors. Our work supports 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 (Terry et al., 2007b). In behavioral studies, haloperidol impaired performance in object recognition and Morris water maze tasks as early as 8-14 days whereas risperidone-treated animals showed slight improvements in Morris water maze tests early during treatment but were impaired in the maze at the longest time point, 174-180 days (Terry et al., 2007b).

These points raise the question of the potential of antipsychotics to further disrupt processes already disturbed in a chronic disease by exacerbating dysfunction of the cholinergic system. The purpose of this work was to determine if long-term treatment

with one representative FGA, haloperidol, or one representative SGA, risperidone, in the rat affect the acquisition of 5-CSRTT and if either drug altered the levels of two cholinergic markers, vesicular acetylcholine transporter (VAChT) or the alpha-7 nicotinic acetylcholine receptor (α7 nAChR), in the medial prefrontal cortex after 320 days of exposure.

METHODS

Animal Care

All procedures employed during this study were reviewed and approved by the Medical College of Georgia Institutional Animal Care and Use Committee and are consistent with AAALAC guidelines. Measures were taken to minimize pain or discomfort in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Significant efforts were also made to minimize the total number of animals used while maintaining statistically valid group numbers.

Male albino Wistar rats (Harlan Sprague-Dawley, Inc.) approximately two months old were housed singly in a temperature controlled room (25^{O}C), maintained on a reversed 12-hour light/dark cycle with free access to water. Food (Teklad Rodent Diet 8604 pellets, Harlan, Madison, WI) was restricted to maintain a target weight of approximately 325 grams. All animals were handled for four days prior to behavioral testing for two to five minutes during each handling session.

Drug administration

Oral antipsychotic dosing was based on previous rodent studies in our laboratory in which time-dependent behavioral and neurochemical effects were detected and plasma drug levels were achieved that approximated those often associated with antipsychotic effects in humans (Terry et al., 2002; Terry et al., 2003; Terry et al., 2005). Furthermore, for both HAL and RISP, the doses selected (see below) would be expected to achieve comparable (and therapeutically) relevant D_2 receptor occupancy values in vivo (i.e., in the range 65-80%; see (Kapur et al., 2003) based on the recent work of (Barth et al., 2006). Rats were thus treated with 1.0 or 2.0 mg/kg/day HAL (Sigma-Aldrich, St. Louis, MO) or 1.25 or 2.5 mg/kg/day risperidone (Pfizer, Inc., New York, NY) orally for a period of 320 days (n=8-10 per treatment group). The antipsychotics were dissolved in 0.1 M acetic acid and subsequently diluted (1:100) with distilled, deionized water for daily drug administration in drinking water. Drug dosing was based on the average daily fluid consumption and the weight of the animals. Animals that were evaluated for residual neuroleptic-related behavioral effects were administered antipsychotics at the doses described above (or vehicle) for 320 days then sacrificed for neurochemical studies.

Behavioral testing

The 5-CSRTT was performed in automated nine-hole operant chambers (Med-Associates, Inc.) housed in sound-insulated and ventilated enclosures. Each SRTT apparatus contains a food magazine on one wall, and on the opposite wall five square niches (i.e., nose-poke holes, 2.5-cm-wide square, 4 cm in depth) arranged on a curved panel and raised 2 cm above the floor. All apertures in the chamber, including the food magazine, are controlled by a photocell monitoring the entrance. Each hole can be

illuminated by a 2.8-W lamp located at the rear of the hole. Each animal has to poke its nose in one of the holes when it is illuminated and then turn around and go to the food magazine to collect a food pellet as a reward.

Training and testing procedures

During each session, the rat is trained to push the food magazine to initiate a trial. Five seconds later, one of the five nose-poke apertures is lit for a specified stimulus length (e.g. 1 s). The rat is then trained to quickly respond with a nose-poke in the hole in which the stimuli was just presented (correct response). The stimuli are presented across the five possible nose-poke apertures in a pseudorandom order. Each correct response is rewarded with a food pellet, and each failure to respond (omissions, longer than 4, 5 or 10 sec post-stimuli presentation depending on the stimulus duration) or incorrect response (response in aperture that was not lit by light stimulus) is punished with a 10 sec time-out with no access to a food pellet. Performance is assessed by both the percentage of correct responses (i.e., number of correct responses/total number of trials, expressed as a percentage) and percentage of omissions (i.e., number of trials without a nose-poke response/total number of trials, expressed as a percentage). Speed of responding is recorded and assessed by both the measure of the time between the onset of the stimulus and the correct response (correct latency) and the time between the correct response and the collection of the food reward (magazine latency). Inappropriate responses include premature responses (responses before stimulus presentation) and perseverative responses (continued response after initial response to stimuli).

Animals (n=8-10 per treatment/vehicle group) started 5CSRTT on day 84 of exposure and moved through a series of five criterion points to complete the task. All subjects had previously been tested in radial arm maze (in press). Subjects were exposed to 100 trials each day five days per week with a session length limited to 30 minutes. The criterion points consisted of 10 second (s) stimulus length and 10 s response time; 5 s stimulus time/4 s response time; 2.5 s stimulus/5 s response time; 1 s stimulus/5 s response time; and 0.5 s stimulus/5 s response time. Criterion was defined as five consecutive days at 90% or higher correct responses for the first three stimulus lengths, but was decreased to 75% or higher for the last two stimulus lengths. Animals were allowed a maximum of 150 days to reach and complete the final criterion phase. If an animal tested for 80 days at any one phase without progressing, it was discontinued from the task, and criterion entered as 80 days. Upon successful completion of the last criterion point, animals were no longer tested but maintained on food restriction until sacrifice.

Dissection

Isofluorane-anesthetized rats were decapitated and the brains were removed from the skulls within 3 min. The brains were immediately frozen in dry ice-cooled methylbutane (isopentane), and then wrapped in aluminum foil before storing at −70 °C. Immediately before each dissection, the rat brain was acclimated from −70 °C to −20 °C for at least 20 min before placing the brain (ventral surface up) in an ice-cold, stainless steel dissection block fabricated for cutting 1 mm wide coronal brain slices. Chilled razor

blades (0.009 in. thick) were inserted into the blocked brain to cut slices that were used in the dissections. All dissections were performed on an ice-chilled, flat glass surface. A scalpel was used to dissect the medial prefrontal cortex region (Palkovits and Brownstein, 1988).

Preparation of brain lysates and following ELISAs were done according to (Gearhart et al., 2006) and (Terry and Gearhart, 2007). Briefly, medial prefrontal cortex was dissected and then homogenized in RIPA buffer containing protease inhibitors and glycerol. The Micro BCA™ Protein Assay Kit (Pierce Biotechnology; Rockford, IL) was used to determine the total protein concentration in each brain lysate. An indirect ELISA was devised to measure the relative levels of VAChT and of α7 nicotinic receptor protein in brain lysates. As an internal control for day-to-day variation in the ELISA methods, brain lysates from vehicle, haloperidol, and risperidone-treated rats were assayed on the same ELISA plate. Brain lysates (10–20 µg total protein/µl) were diluted in sodium carbonate buffer (pH 9.6), and then the diluted lysates (0.2 µg50 µl/well VAChT and 0.2 µg and 0.4 µg protein/50 µl/well for α7 nAChR) were adsorbed to Nunc Maxisorp TM ELISA plates (overnight at 5 °C). Microwells were rinsed once with 300 µl of wash buffer (pH 7.4 phosphate-buffered saline containing 0.05% (v/v) Triton X-100), before blocking the wells for one hour with 300 µl/well 1% (w/v) nonfat dry milk in PBST.

Wells were rinsed with wash buffer (300 µl/well), before adding either 50 µl/well of VAChT antibody (Sigma #V-5387; rabbit polyclonal; diluted 1:100 in blocking buffer) or 50 µl/well of α7 nicotinic receptor antibody (Chemicon AB5637 rabbit polyclonal diluted 1:100 in blocking buffer for α7 nAChR). Incubation with primary antibody was carried out at room temperature for two hours, and then the microwells were rinsed with wash

buffer (five times, 300 µl/well). The primary antibodies for VAChT and α7 nAChR were detected using peroxidase (HRP)-conjugated goat anti-rabbit IgG (Jackson Immunoresearch #115-035-144, diluted 1:10,000 in blocking buffer, 50 µl/well). After one hour at room temperature, the microplate was rinsed five times with wash buffer (300 µl/well). Tetramethylbenzidine (100 µl/well, KPL 50-76-01) was the peroxidase substrate; after 15–30 min at room temperature, 1 M HCl (100 µl/well) was added to stop the peroxidase reaction (color changed from blue to yellow). The absorbance of the yellow reaction product was measured at 450 nm using a microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT).

Statistical Analyses

5CSRTT

To examine performance across the first 10 days of testing at the 10 seconds stimulus duration, two-way repeated measures ANOVA was used. To examine differences in various continuous measures between treatment groups within delay controlling for the number of days to acquire the task, analysis of covariance was used (ANCOVA). Post hoc multiple comparisons between treatment groups were performed using a Bonferroni adjustment. To examine differences in the proportion acquiring the task within delay between treatment groups, chi-square, or Fisher exact if the assumptions to the chi-square were violated, tests were used. Differences in the number of days to acquire the task between treatment groups within delay were examined using survival analysis and a Cox-Mantel log-rank test. All statistical analyses were performed using SAS 9.1.3. Statistical significance was assessed using an alpha level of 0.05.

ELISAs

Statistical analyses were made using a one-way analysis of variance (ANOVA). Student Knewman Keuls procedure was used to examine post hoc differences (p value < 0.05 considered significant).

RESULTS

5-CSRTT

There was a treatment effect on the percentage of animals achieving each criterion point as seen in Table 4.1. Significantly fewer animals treated with 2.0 HAL achieved criterion at the 10 s stimulus duration than vehicle animals ($p<0.0001$). At the 5s stimulus duration, significantly fewer animals treated with 2.5 RIS, 1.0 HAL, and 2.0 HAL successfully moved to next trial block ($p<0.0001$) relative to vehicle animals. At the 2.5 and 1.0 stimulus duration, all treatment groups had significantly less animals making criterion than vehicle ($p<0.0001$). All treatment groups except 1.25 RIS had fewer animals to complete the final and shortest stimulus duration and thus, complete the task in its entirety, than vehicle ($p<0.0001$).

10 Second Stimulus Duration

At the 10 s stimulus duration (fig. 4.1), there was a significant difference in trials to criterion ($X^2_{(4)}=26.8146$, $p<0.0001$) among treatment groups. Results of trials for first 10 days of training for the 10 s stimulus delay are shown in Figures 4.1 and 4.2. The 2.0 and 1.0 HAL groups required significantly more trials than vehicle animals to reach criterion. There was a significant day effect on percent of correct responses, percent omissions, premature responses, perseverative responses, correct latencies, incorrect

latencies, and latencies to reward with animals improving performance across daily sessions. Treatment had a significant effect on percent of correct responses ($F_{(4,35)}$= 5.363, p=0.0002). Further analyses revealed 2.0 HAL animals had fewer correct responses than vehicle subjects. A significant treatment by day effect was found in this measure ($F_{(36,315)}$= 1.495, p=0.039). Haloperidol treated animals had scored less percent correct than vehicles on half of the days for the 2.0 dose and one day for the 1.0 dose. There was also a significant treatment effect on number of omissions ($F_{(4,35)}$=6.28, p<0.001). Post hoc analyses showed 1.0 and 2.0 HAL animals had more omissions than vehicle animals. A significant treatment by day effect was found in this measure ($F_{(36,315)}$= 1.759, p=0.006). All groups had more omissions than vehicle animals on at least one day and 2.0 Hal animals had significantly more on eight of ten days. There was a significant effect of treatment on anticipatory or premature responses ($F_{(4,35)}$= 3.696, p=0.0013). Further analyses revealed 2.0 HAL and 1.25 RIS animals had fewer premature responses than vehicle subjects. No significant treatment by day interaction was detected in this measure. There was also a significant effect of treatment on perseverative responses ($F_{(4,35)}$= 25.498, p<0.001). Further analyses revealed all treatment groups had more perseverative responses than vehicle-treated subjects. A significant treatment by day effect was found in this measure ($F_{(36,315)}$= 1.605, p=0.019). Again, all groups had significantly more perseverative responses than vehicle animals across days.

Additional treatment effects were found in the three different latency measures. Treatment had a significant effect on mean latency to correct responses ($F_{(4,35)}$= 10.542,

p<0.001). Further analyses revealed all treatment groups took more time to make a correct response than vehicle subjects. A significant treatment by day effect was found in this measure ($F_{(36,315)}$= 2.596, *p*<0.001). Again, all groups had significantly more perseverative responses than vehicle animals across most days. There was also a significant treatment effect on mean latency to incorrect responses ($F_{(4,35)}$=4.564, *p*<0.005). Post hoc analyses showed all groups except 1.25 RIS had longer latencies to incorrect responses than vehicle animals. A significant treatment by day effect was found in this measure ($F_{(36,315)}$= 1.712, *p*=0.009). Both HAL groups and the higher dose RIS group had longer incorrect response times than vehicle subjects. Treatment had a significant effect on mean latency to reward ($F_{(4,35)}$= 26.593, *p*<0.001). Further analyses revealed all treatment groups except 1.25 RIS took more time to collect reward pellets than vehicle subjects. A significant treatment by day effect was found in this measure ($F_{(36,315)}$= 5.896, *p*<0.001). Again, both HAL groups and the higher dose RIS group had significantly took significantly longer to collect reward pellets than vehicle animals across most days.

5 Second Stimulus Duration

At the 5 s stimulus duration (fig. 4.1), there was a significant difference in trials to criterion ($X^2_{(4)}$=52.6094, *p*<0.0001) across the various treatment groups. All groups receiving antipsychotics required significantly more trials to achieve criterion than vehicle. There was a trend toward a treatment effect ($F_{(4,30)}$=2.40, *p*=0.0717)on number of omissions (fig. 4.4A). Post hoc analysis indicated more omissions in the HAL 2.0-treamtent group than vehicle. There was not a significant difference at this stimulus

duration in the number of premature responses (fig. 4.4B), however perseverative responses (fig. 4.4C) did significantly differ ($F_{(4,30)}$= 3.00, p=0.0338). Post hoc analysis revealed animals treated with 1.0 and 2.0 HAL (p<0.1) perseverated more than vehicle subjects. Treatment groups did not differ in the average latency to correct responses (fig. 4.5A) or incorrect responses (fig. 4.5B), and there was also not a difference in average latency to reward (fig. 4.5C).

2.5 Second Stimulus Duration

At the 2.5 s stimulus duration (fig. 4.1), there was a significant difference in trials to criterion ($X^2_{(3)}$=10.0285, p=0.0183). Post hoc analysis revealed that all antipsychotic treatment groups required more trials than vehicle animals. There was trend toward a treatment effect ($F_{(3,19)}$=2.84, p=0.0656) on omissions (fig. 4.4A). Post hoc analysis showed both 1.25 (p=0.0465) and 2.5 RIS (p=0.0382) treated animals omitted more trials than vehicles. There was a significant difference at this stimulus duration in the number of premature responses ($F_{(3,19)}$= 4.36, p=0.017) as shown in figure 4.4B. Post hoc analysis showed differences among treatment groups but not in comparison to vehicle. Perseverative responses did not significantly differ (fig. 4.4C). Again, the groups did not differ in the average latency to correct responses (fig. 4.5A) or incorrect responses (fig. 4.5B), although there was a difference in average latency to reward ($F_{(3,19)}$=7.68, p=0.0015) as seen in figure 4.5C. According to post hoc analysis, 1.0 HAL (p=0.002) and 2.5 RIS (p=0.0009) animals were slower to collect the reward than vehicle animals.

1.0 Second Stimulus Duration

At the 1.0 s stimulus duration (fig. 4.1), there was a trend in trials to criterion ($X^2_{(3)}$=7.0099, *p*=0.0716) showing 2.5 RIS animals requiring more trials than vehicle. There was also no significant treatment effect on number of omissions (fig. 4.4A). At this stimulus duration, there was not a significant difference in the number of premature responses (fig. 4.4B) or perseverative responses (fig. 4.4C). Treatment groups did not differ in the average latency to correct responses (fig. 4.5A) or incorrect responses (fig. 4.5B), although there was a difference in average latency to reward ($F_{(3,19)}$=7.91, *p*=0.0013) as seen in figure 4.5C. According to post hoc analysis, 2.5 RIS animals were slower to collect the reward than vehicle animals (*p*=0.0002).

0.5 Second Stimulus Duration

At the 0.5 s stimulus duration (fig. 4.1), there was no difference in trials to criterion ($X^2_{(3)}$=1.8013, *p*=0.4063). There was also no significant treatment effect on number of omissions (fig. 4.4A). However, there was a significant difference at the shortest stimulus duration in the number of premature responses ($F_{(3,16)}$= 10.31, *p*=0.0005) as seen in figure 4.4B. Post hoc analysis showed 1.0 Hal animals had more premature responses than vehicles *(p*<0.0001). Perseverative responses (fig. 4.4C) also significantly differed at this stimulus duration ($F_{(3,16)}$= 3.99, *p*=0.0268). Post hoc analysis revealed animals treated with 2.5 RIS perseverated more than vehicle subjects (*p*=0.0078). Treatment groups did not differ in the average latency to correct responses (fig. 4.5A) or incorrect responses (fig. 4.5B), although there was a difference in average latency to reward ($F_{(3,16)}$=3.87, *p*=0.0294) as seen in figure 4.5C. According to post hoc

analysis, 2.5 RIS animals were slower to collect the reward than vehicle animals (p=0.0006).

ELISAs

Results from ELISA data are shown in figure 4.6. There were no significant treatment effects on amounts of α7 nAChR found in medial prefrontal cortex. Likewise, there were no significant treatment effects on amounts of VAChT detected in medial prefrontal cortex.

DISCUSSION

The results of the experiments conducted in this study can be summarized as follows: 1) In 5CSRTT experiments haloperidol and, to a lesser degree, risperidone, impaired task acquisition as indicated by the failure (or increase in the number of trials) to meet specific performance criteria. 2) Haloperidol treated animals failed to fully acquire the task whereas risperidone-treated animals performed more similarly to vehicle animals, but were generally impaired as the task became more difficult. 3) At both the longer and shorter stimulus durations both antipsychotics generally increased the number of perseverative responses as well as latencies to respond to stimuli and collect rewards. 4) Haloperidol and risperidone were without significant effect in the subsequent ELISA experiments which were conducted to detect potential (antipsychotic-related) alterations in the levels of VAChT and α7 nAChR in medial prefrontal cortex of rats treated for 320 days. These results therefore, lead to several possible conclusions. First, chronic exposure to therapeutic doses of haloperidol and risperidone appears to impair the

acquisition of a task of sustained attention, vigilance, and executive function. Given the general increases in response and reward latencies across the study (i.e., in all of the antipsychotic-treated groups), such impairments in 5C-SRTT acquisition may in part be due to alterations in signal detection, psychomotor speed, and/or reward motivation. An increase in number of perseverative responses is another factor that could alter performance of the task (i.e. by increasing compulsive-type behaviors). Finally, the behavioral impairments do not appear to be due to deficits in VAChT or the α7 nAChR in medial prefrontal cortex, but may instead be due to either alterations of other cholinergic proteins or the modulation of the dopamine or serotonin neurotransmitter systems.

Our results generally support other studies where haloperidol was found to disrupt sustained attention in rats by increasing latencies to response and to reward as well as increasing the number of omissions (Brockel and Fowler, 1995). In addition, acute treatment with both haloperidol and risperidone impaired sustained attention in an operant visual signal detection task. (Rezvani and Levin, 2004) Such effects are not thought to be associated with motor impairment as Skjoldager and Fowler (1991) found haloperidol treated rats had increased errors of omission and reaction time in a sustained attention task designed for less movement than required by 5-CSRTT.

As in our animal study, clinical studies have shown that antipsychotics affect CPT performance but also specific components of the CPT as well. Morrens et. al. (2007) found even short-term administration of haloperidol in healthy volunteers impairs pursuit task performance requiring simultaneous visuospatial monitoring, sensorimotor speed,

and accuracy without sedative effects on other psychomotor skills. In addition, on psychomotor driving tests, patients receiving typical antipsychotics scored worse than the general population as well as patients receiving atypical drugs especially on measures of attention and divided attention. (Brunnauer et al., 2004). In a similar study, schizophrenics treated with risperidone or haloperidol both scored lower on the same psychomotor driving tests, but haloperidol patients showed greater impairments than those using risperidone (Soyka et al., 2005). Furthermore, another study revealed risperidone and its metabolite 9OH risperidone both disrupted CPT performance (Chen et al., 2004). Similar to our studies, plasma levels of the compounds inversely related to CPT performance. In yet another study, schizophrenics receiving FGA therapy and normal controls were tested with CPT as a baseline measure (Harvey et al., 2000). When half of the treated patients were switched to SGA therapy, their CPT scores improved and were better at the end of the study than their FGA counterparts. In a large study, first episode patients treated with either haloperidol or risperidone experienced improvements from baseline in episodic memory, verbal fluency, vigilance, and visuomotor speed but only patients receiving risperidone improved in executive function (Harvey et al., 2005). After three months of antipsychotic drug exposure, Harvey concluded risperidone was overall more beneficial than haloperidol. In contrast, Liu et.al. (2000) found no differences in CPT performance between baseline and posttests after 12 weeks of either with either risperidone or haloperidol.

In regard to the VAChT and α7 nAChR ELISAs, the results would indicate that the cholinergic system as it relates to these two markers in the medial prefrontal cortex

region, is not disrupted by haloperidol or risperidone in rats treated for 320 days. Data previously published by our laboratory did find differences in VAChT (Terry et al., 2007a) and α7 nAChR (Terry and Gearhart, 2007) in prefrontal cortex; however, these previous data were derived from samples containing all prefrontal cortex instead of only medial prefrontal cortex. Also, the earlier tests were performed on subjects with much shorter exposure periods (90 days), and the extended exposure period may allow time for some compensatory mechanism to restore the presence of these proteins in the brain. Interestingly, through cholinergic antagonist studies in rats, other investigators conclude that it is actually the modulation of $\alpha4\beta2$, $\alpha4\beta4$ or $\alpha3\beta2$ nAChRs, not α7 nAChRs, that result in improved 5CSRTT scores after nicotine administration (Blondel et al., 2000; Grottick et al., 2003). In either case, nicotine administration in human studies involving smokers after one night of abstinence has been found to reverse deficient scores on Conners CPT imposed by haloperidol (Levin et al., 1996) and, in a similar study, to improve scores in patients receiving SGAs as their regular therapy (Smith et al., 2006). Haloperidol induced impairment has also been partially corrected in a rat model for sustained attention as well (Rezvani and Levin, 2004).

Singling out the cholinergic system as unilateral modulator of 5CSRTT or CPT would be an oversimplification of both neuronal and global brain processes. Dopamine affects not only 5CSRTT performance but response to nicotine imposed improvements as well (Hahn et al., 2002). Antagonism of 5HT2A serotonin receptors decreases premature responses and increases accuracy (Fletcher et al., 2007). Studies with acute administration of phencyclidine, an NMDA antagonist, resulted in poorer performance

on 5CSRTT that is further disrupted with concomitant administration of acute risperidone or clozapine (Amitai et al., 2007). Zmarowski (2007) actually found D1 receptor regulation of NMDA receptors in the nucleus accumbens is in part responsible for the modulation of cortical acetylcholine, affecting attentional processes and motor responses to stimuli.

In conclusion, the results of this study indicate that chronic exposure to therapeutic doses of haloperidol and risperidone (to a lesser degree) leads to impairments in the acquisition of a task of sustained attention, vigilance, and executive function. Such impairments may in part be due to alterations in signal detection, psychomotor speed, and reward motivation. The behavioral impairments do not appear to be due to deficits in VAChT or the α7 nAChR in medial prefrontal cortex, but may instead be due to either alterations of other cholinergic proteins and receptors or due to modulation other neurotransmitter systems. Further, although haloperidol impairments were apparent early in testing (i.e., during the less difficult sessions), risperidone may impair attention and executive function as the demands of the task increase.

AKNOWLEDGEMENTS

This work was supported in part by the American Foundation for Pharmaceutical Education's Pre-doctoral Fellowship program and by the National Institute of Mental Health (MH 066233 to AVT).

REFERENCES

Amitai N., Semenova S., Markou A. 2007. Cognitive-disruptive effects of the psychotomimetic phencyclidine and attenuation by atypical antipsychotic medications in rats. *Psychopharmacology (Berl) 193*, 521-537.

Barth V.N., Chernet E., Martin L.J., Need A.B., Rash K.S., Morin M., Phebus L.A. 2006. Comparison of rat dopamine D2 receptor occupancy for a series of antipsychotic drugs measured using radiolabeled or nonlabeled raclopride tracer. *Life Sci 78*, 3007-3012.

Beck L.H., Bransome E.D., Jr., Mirsky A.F., Rosvold H.E., Sarason I. 1956. A continuous performance test of brain damage. *J Consult Psychol 20*, 343-350.

Blondel A., Sanger D.J., Moser P.C. 2000. Characterisation of the effects of nicotine in the five-choice serial reaction time task in rats: antagonist studies. *Psychopharmacology (Berl) 149*, 293-305.

Brockel B.J., Fowler S.C. 1995. Effects of chronic haloperidol on reaction time and errors in a sustained attention task: partial reversal by anticholinergics and by amphetamine. *J Pharmacol Exp Ther. 275*, 1090-1098.

Brunnauer A., Laux G., Geiger E., Moller H.J. 2004. The impact of antipsychotics on psychomotor performance with regards to car driving skills. *J Clin Psychopharmacol 24*,155-160.

Carli M., Robbins T.W., Evenden J.L., Everitt B.J. 1983. Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behav Brain Res 9*, 361-380.

Chen P.S., Yang Y.K., Su S.-F., Liao Y.-C., Chang J.-W., Yeh T.L. 2004. Correlation between scores on Continuous Performance Test and plasma concentration for schizophrenic patients on risperidone. *Psychiatry Clin Neurosci 58,* 168-172.

Chudasama Y., Muir J.L. 2001. Visual attention in the rat: a role for the prelimbic cortex and thalamic nuclei? *Behav Neurosci 115*, 417-428.

Chudasama Y., Passetti F., Rhodes S.E., Lopian D., Desai A., Robbins T.W. 2003. Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity. *Behav Brain Res 146*, 105-119.

Correll C.U., Leucht S., Kane J.M. 2004. Lower Risk for Tardive Dyskinesia Associated With Second-Generation Antipsychotics: A Systematic Review of 1-Year Studies. *American Journal of Psychiatry 161*, 414-425.

Crook J.M., Tomaskovic-Crook E., Copolov D.L., Dean B. 2000. Decreased muscarinic receptor binding in subjects with schizophrenia: a study of the human hippocampal formation. *Biol Psychiatry 48*, 381-388.

Crook J.M., Tomaskovic-Crook E., Copolov D.L., Dean B. 2001. Low muscarinic receptor binding in prefrontal cortex from subjects with schizophrenia: a study of Brodmann's areas 8, 9, 10, and 46 and the effects of neuroleptic drug treatment. *Am J Psychiatry 158*, 918-925.

Dalley J.W., Cardinal R.N., Robbins T.W. 2004a. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav Rev 28*, 771-784.

Dalley J.W., Theobald D.E., Bouger P., Chudasama Y., Cardinal R.N., Robbins T.W. 2004b. Cortical cholinergic function and deficits in visual attentional performance in rats following 192 IgG-saporin-induced lesions of the medial prefrontal cortex. *Cereb Cortex 14,* 922-932.

Davies L.M., Lewis S., Jones P.B., Barnes T.R.E., Gaughran F., Hayhurst K., Markwick A., Lloyd H. 2007. Cost-effectiveness of first- v. second-generation antipsychotic drugs: results from a randomised controlled trial in schizophrenia responding poorly to previous therapy. *Curr Psychiatry 191*, 14-22.

Dean B., Crook J.M., Opeskin K., Hill C., Keks N., Copolov D.L. 1996. The density of muscarinic M1 receptors is decreased in the caudate-putamen of subjects with schizophrenia. *Mol Psychiatry 1*, 54-58.

Fletcher P.J., Tampakeras M., Sinyard J., Higgins G.A. 2007. Opposing effects of 5-HT(2A) and 5-HT (2C) receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test. *Psychopharmacology (Berl) 3*:3.

Friedman J.I. 2004. Cholinergic targets for cognitive enhancement in schizophrenia: focus on cholinesterase inhibitors and muscarinic agonists. *Psychopharmacology (Berl) 174*, 45-53.

Gearhart D.A., Middlemore M.-L., Terry A.V. 2006. ELISA methods to measure cholinergic markers and nerve growth factor receptors in cortex, hippocampus, prefrontal cortex, and basal forebrain from rat brain. *Journal of Neuroscience Methods 150*, 159-173.

Green M.F. 1996. What are the functional consequences of neurocognitive deficits in schizophrenia? *Am J Psychiatry 153*, 321-330.

Grottick A.J., Haman M., Wyler R., Higgins G.A. 2003. Reversal of a vigilance decrement in the aged rat by subtype-selective nicotinic ligands.

Neuropsychopharmacology 28, 880-887.

Hahn B., Shoaib M., Stolerman I.P. 2002. Effects of dopamine receptor antagonists on nicotine-induced attentional enhancement. *Behav Pharmacol 13*, 621-632.

Harvey P.D., Moriarty P.J., Serper M.R., Schnur E., Lieber D. 2000. Practice-related improvement in information processing with novel antipsychotic treatment. *Schizophr Res 46*,139-148.

Harvey P.D., Rabinowitz J., Eerdekens M., Davidson M. 2005. Treatment of Cognitive Impairment in Early Psychosis: A Comparison of Risperidone and Haloperidol in a Large Long-Term Trial. *Am J Psychiatry 162*, 1888-1895.

Jones D.N., Barnes J.C., Kirkby D.L., Higgins G.A. 1995. Age-associated impairments in a test of attention: evidence for involvement of cholinergic systems. *J Neurosci 15*, 7282-7292.

Kapur S., VanderSpek S.C., Brownlee B.A., Nobrega J.N. 2003. Antipsychotic Dosing in Preclinical Models Is Often Unrepresentative of the Clinical Condition: A Suggested Solution Based on in Vivo Occupancy. *J Pharmacol Exp 305*, 625-631.

Keefe R.S., Bilder R.M., Davis S.M., Harvey P.D., Palmer B.W., Gold J.M., Meltzer H.Y., Green M.F., Capuano G., Stroup T.S. McEvoy J.P., Swartz M.S., Rosenheck R.A., Perkins D.O., Davis C.E., Hsiao J.K. Lieberman J.A. 2007. Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE Trial. *Arch Gen Psychiatry 64*, 633-647.

Kurtz M.M., Moberg P.J., Ragland J.D., Gur R.C., Gur R.E. 2005. Symptoms Versus Neurocognitive Test Performance as Predictors of Psychosocial Status in Schizophrenia: A 1- and 4-Year Prospective Study. *Schizophrenia Bull 31*, 167-174.

Levin E.D., Wilson W., Rose J.E., McEvoy J. 1996. Nicotine-haloperidol interactions and cognitive performance in schizophrenics. *Neuropsychopharmacology 15*, 429-436.

Liu S.K., Chen W.J., Chang C.J., Lin H.N. 2000. Effects of atypical neuroleptics on sustained attention deficits in schizophrenia: a trial of risperidone versus haloperidol. *Neuropsychopharmacology 22*, 311-319.

Mackworth J.F., Taylor M.M. 1963. The D' Measure of Signal Detectability in Vigilance-Like Situations. *Can J Psychol 17*, 302-325.

Morrens M., Wezenberg E., Verkes R.J., Hulstijn W., Ruigt G.S., Sabbe B.G. 2007. Psychomotor and memory effects of haloperidol, olanzapine, and paroxetine in healthy subjects after short-term administration. *J Clin Psychopharmacol 27*, 15-21.

Muir J.L., Everitt B.J., Robbins T.W. 1995. Reversal of visual attentional dysfunction

following lesions of the cholinergic basal forebrain by physostigmine and nicotine but not by the 5-HT3 receptor antagonist, ondansetron. *Psychopharmacology (Berl) 118*, 82-92.

Nuechterlein K.H., Barch D.M., Gold J.M., Goldberg T.E., Green M.F., Heaton R.K. 2004. Identification of separable cognitive factors in schizophrenia. *Schizophr Res 72*, 29-39.

Palkovits M., Brownstein M.J. 1988. Maps and guide to microdissection of the rat brain. New York: Elsevier.

Passetti F., Chudasama Y., Robbins T.W. 2002. The Frontal Cortex of the Rat and Visual Attentional Performance: Dissociable Functions of Distinct Medial Prefrontal Subregions. *Cerebral Cortex 12*, 1254-1268.

Passetti F., Dalley J.W., O'Connell M.T., Everitt B.J., Robbins T.W. 2000. Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. *The European Journal Of Neuroscience 12*, 3051-3058.

Pinkham A.E., Penn D.L. 2006. Neurocognitive and social cognitive predictors of interpersonal skill in schizophrenia. *Psychiatry Res 143*, 167-178.

Powchik P., Davidson M., Haroutunian V., Gabriel S.M., Purohit D.P., Perl D.P., Harvey P.D., Davis K.L. 1998. Postmortem studies in schizophrenia. *Schizophr Bull 24*, 325-341.

Rezvani A.H., Levin E.D. 2004. Nicotine-antipsychotic drug interactions and attentional performance in female rats. *Eur J Pharmacol 486*, 175-182.

Risbrough V., Bontempi B., Menzaghi F. 2002. Selective immunolesioning of the basal forebrain cholinergic neurons in rats: effect on attention using the 5-choice serial reaction time task. *Psychopharmacology (Berl) 164*,71-81.

Robbins T.W. 2002. The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology (Berl) 163*, 362-380.

Rosenheck R.A. 2007. Evaluating the cost-effectiveness of reduced tardive dyskinesia with second-generation antipsychotics. *The British J of Psychiatry 191*, 238-245.

Shirzadi A.A., Ghaemi S.N. 2006. Side Effects of Atypical Antipsychotics: Extrapyramidal Symptoms and the Metabolic Syndrome. *Harvard Rev of Psychiatry 14*, 152 – 164.

Skjoldager P., Fowler S.C. 1991. Scopolamine attenuates the motor disruptions but not the attentional disturbances induced by haloperidol in a sustained attention task in the rat. *Psychopharmacology (Berl) 105*, 93-100.

Smith R.C., Warner-Cohen J., Matute M., Butler E., Kelly E., Vaidhyanathaswamy S., Khan A. 2006. Effects of nicotine nasal spray on cognitive function in schizophrenia. *Neuropsychopharmacology 31*, 637-643.

Soyka M., Winter C., Kagerer S., Brunnauer M., Laux G., Moller H.J. 2005. Effects of haloperidol and risperidone on psychomotor performance relevant to driving ability in schizophrenic patients compared to healthy controls. *J Psychiatr Res 39*,101-108.

Terry A.V., Jr., Gearhart D.A. 2007. Time dependent decreases in central alpha7 nicotinic acetylcholine receptors associated with haloperidol and risperidone treatment in rats. *Eur J Pharmacol 571*, 29-32.

Terry A.V., Jr., Gearhart D.A., Mahadik S.P., Warsi S., Davis L.W., Waller J.L. 2005. Chronic exposure to typical or atypical antipsychotics in rodents: temporal effects on central alpha7 nicotinic acetylcholine receptors. *Neuroscience 136*, 519-529.

Terry A.V., Jr., Gearhart D.A., Warner S., Hohnadel E.J., Middlemore M.L., Zhang G., Bartlett M.G., Mahadik S. P. 2007a. Protracted effects of chronic oral haloperidol and risperidone on nerve growth factor, cholinergic neurons, and spatial reference learning in rats. *Neuroscience* Sept 14.Epub ahead of print..

Terry A.V., Jr., Gearhart D.A., Warner S.E., Zhang G., Bartlett M.G., Middlemore M.L., Beck W.D., Jr., Mahadik S.P., Waller J.L. 2007b. Oral haloperidol or risperidone treatment in rats: temporal effects on nerve growth factor receptors, cholinergic neurons, and memory performance. *Neuroscience 146*, 1316-1332.

Terry A.V., Jr., Hill W.D., Parikh V., Evans D.R., Waller J.L., Mahadik S.P. 2002. Differential effects of chronic haloperidol and olanzapine exposure on brain cholinergic markers and spatial learning in rats. *Psychopharmacology (Berl) 164*, 360-368.

Terry A.V., Jr., Hill W.D., Parikh V., Waller J.L., Evans D.R., Mahadik S.P. 2003. Differential effects of haloperidol, risperidone, and clozapine exposure on cholinergic markers and spatial learning performance in rats. *Neuropsychopharmacology 28*, 300-309.

Uylings H.B., Groenewegen H.J., Kolb B. 2003. Do rats have a prefrontal cortex? *Behav Brain Res 146*, 3-17.

Villalta-Gil V., Vilaplana M., Ochoa S., Haro J.M., Dolz M., Usall J., Cervilla J. 2006. Neurocognitive performance and negative symptoms: are they equal in explaining disability in schizophrenia outpatients? *Schizophr Res 87*, 246-253.

Zmarowski A., Sarter M., Bruno J.P. 2007. Glutamate receptors in nucleus accumbens mediate regionally selective increases in cortical acetylcholine release. *Synapse 61*, 115-123.

TABLE 4.1

Group	n	10s	5s	2.5s	1.0s	0.5s
VEH	**10**	**100**	**100**	**100**	**100**	**60**
1.25 RIS	**8**	**100**	**88**	***75**	***75**	**50**
2.5 RIS	**8**	**100**	***75**	***75**	***75**	***38**
1.0 HAL	**8**	**89**	***33**	***22**	***11**	***0**
2.0 HAL	**8**	***25**	***0**	***0**	***0**	***0**

FIGURE LEGENDS

Fig 4.1. Haloperidol and risperidone (to a lesser degree) impairs acquisition of a task of sustained attention in rats. Rats were trained to meet certain performance criterion at each of the stimulus durations illustrated above. Testing began at 84 days of drug exposure. Each bar represents the mean +/- SEM for each test group. * =Significantly different than vehicle controls (P<0.05). #=trend toward significance (P<0.1). N=8-10 rats/group.

Fig. 4.2. Haloperidol and risperidone (to a lesser degree) impairs acquisition of a task of sustained attention in rats as seen in the first 10 days of training at the 10 second stimulus duration. Testing began at 84 days of drug exposure. Dosing was in mg/kg/day. Each bar represents the mean +/- SEM for each test group. * =Significantly different than vehicle controls (P<0.05). #=trend toward significance (P<0.1). N=8-10 rats/group. VEH=vehicle HAL=haloperidol RISP=Risperidone

Fig. 4.3. Haloperidol and risperidone (to a lesser degree) impairs acquisition of a task of sustained attention in rats as seen in the first 10 days of training at the 10 second stimulus duration. Testing began at 84 days of drug exposure. Dosing was in mg/kg/day. Each bar represents the mean +/- SEM for each test group. * =Significantly different than vehicle controls (P<0.05). #=trend toward significance (P<0.1). N=8-10 rats/group. VEH=vehicle HAL=haloperidol RISP=Risperidone

Fig. 4.4. Haloperidol and risperidone (to a lesser degree) impairs acquisition of a task of sustained attention in rats as seen in the 5, 2.5, 1.0 and 0.5 s stimulus durations. Testing began at 84 days of drug exposure. Each bar represents the mean +/- SEM for each test group. * =Significantly different than vehicle controls (P<0.05). #=trend toward

significance (*P*<0.1). *N*=8-10 rats/group.

Fig. 4.5. Haloperidol and risperidone (to a lesser degree) impairs acquisition of a task of sustained attention in rats as seen in the 5, 2.5, 1.0 and 0.5 s stimulus durations. Testing began at 84 days of drug exposure. Each bar represents the mean +/- SEM for each test group. * =Significantly different than vehicle controls (*P*<0.05). #=trend toward significance (*P*<0.1). *N*=8-10 rats/group.

Fig. 4.6. Effects of chronic treatment with haloperidol or risperidone (compared with vehicle controls) on levels of the nAChR (left) and VAChT (right). For each ELISA, samples from one brain region for each treatment group were analyzed in the same 96-well ELISA plate, and equal amounts of total protein were analyzed across treatment groups. Data are expressed as relative levels (absorbance at 450 nm). There were no significant differences (i.e. *P*< 0.05). *N*=6. VEH=vehicle HAL=haloperidol RIS=Risperidone

TABLE LEGEND

TABLE 4.1. Haloperidol and risperidone (to a lesser degree) impairs acquisition of a task of sustained attention in rats. Dosing was in mg/kg/day. Testing began at 84 days of drug exposure. Rats were trained to meet certain performance criterion at each of the stimulus durations presented in table. Numbers represent the percent of animals from each group that achieved the criterion for that stimulus duration. *=significantly different from vehicle performance ($p<0.05$). VEH=vehicle HAL=haloperidol RIS=Risperidone

Fig 4.1

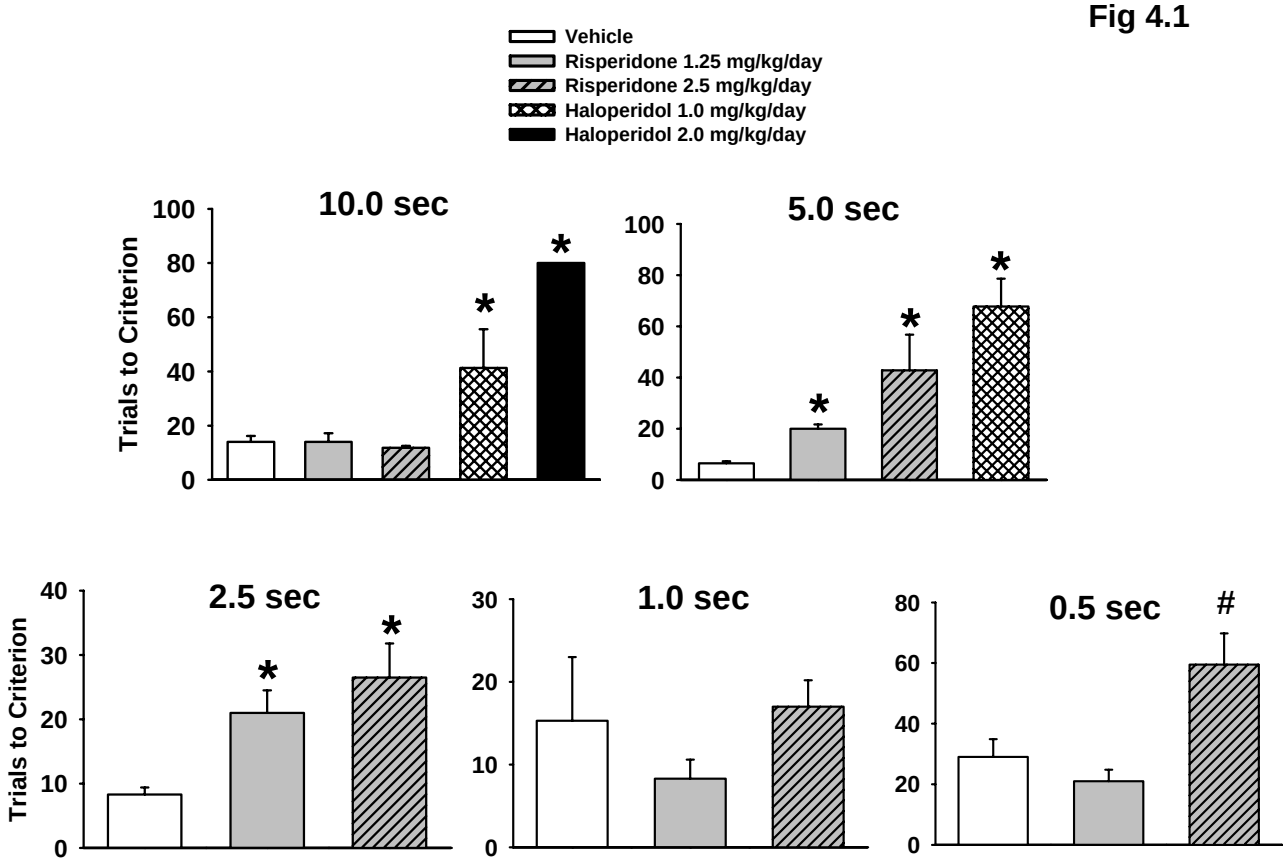

Vehicle
Risperidone 1.25 mg/kg/day
Risperidone 2.5 mg/kg/day
Haloperidol 1.0 mg/kg/day
Haloperidol 2.0 mg/kg/day
10.0 sec
5.0 sec
2.5 sec
1.0 sec
0.5 sec
Trials to Criterion
Trials to Criterion
*
#

Fig 4.2

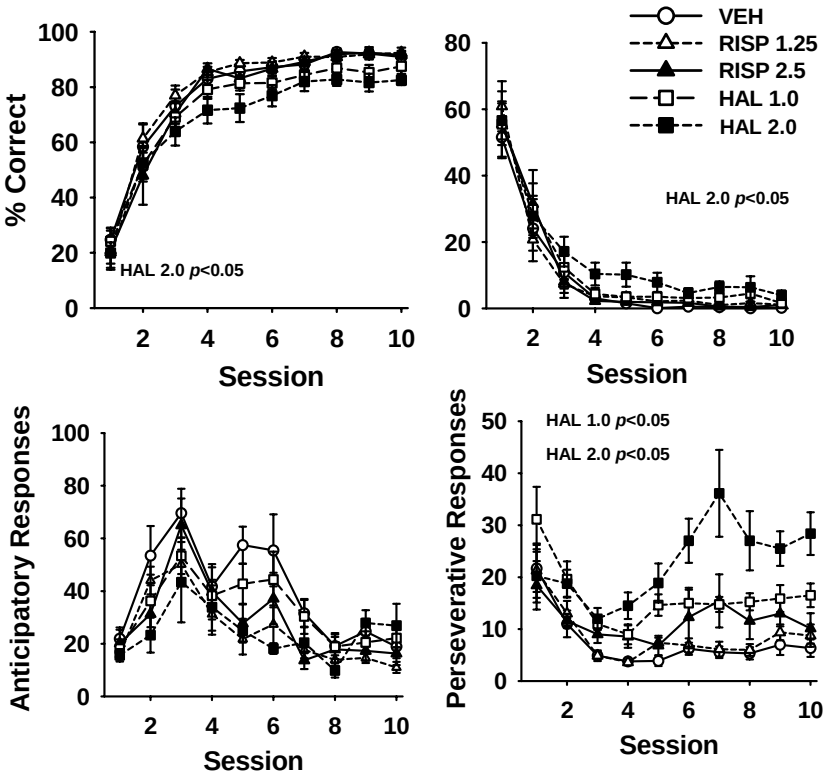

% Correct
HAL 2.0 p<0.05
Session
VEH
RISP 1.25
RISP 2.5
HAL 1.0
HAL 2.0
HAL 2.0 p<0.05
Session
Anticipatory Responses
Session
Perseverative Responses
HAL 1.0 p<0.05
HAL 2.0 p<0.05
Session

Fig 4.3

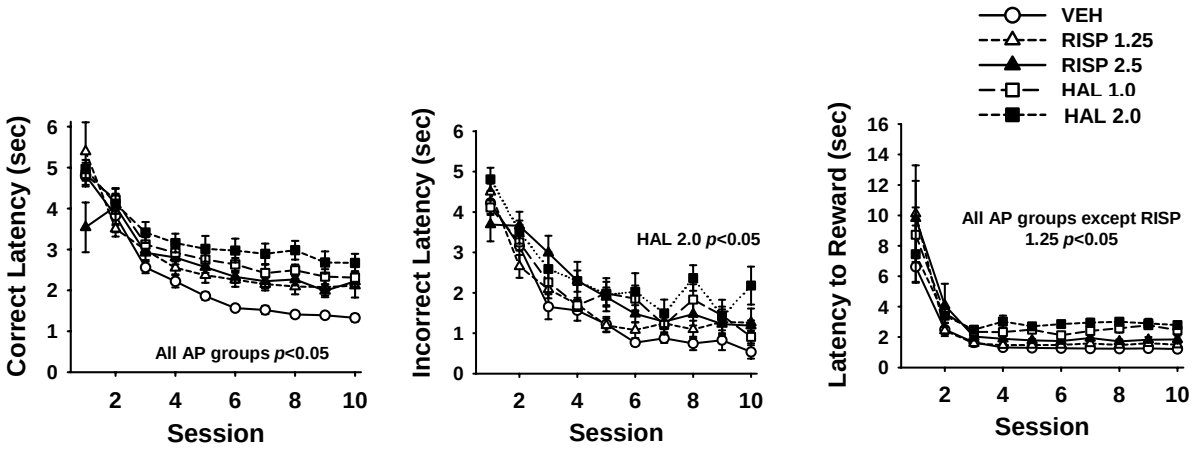

Correct Latency (sec)
All AP groups p<0.05
Session
Incorrect Latency (sec)
HAL 2.0 p<0.05
Session
Latency to Reward (sec)
All AP groups except RISP 1.25 p<0.05
Session
VEH
RISP 1.25
RISP 2.5
HAL 1.0
HAL 2.0

Fig 4.4

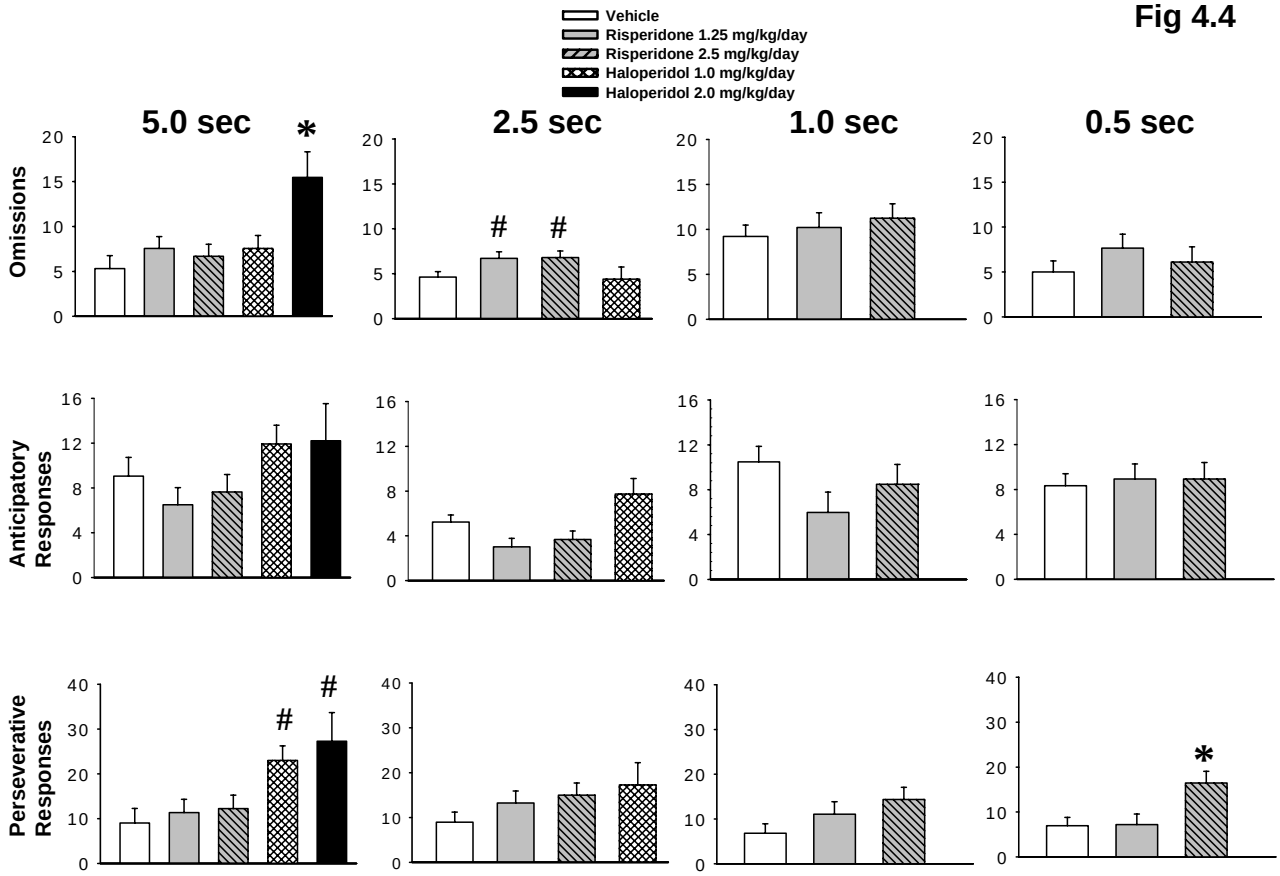

Vehicle
Risperidone 1.25 mg/kg/day
Risperidone 2.5 mg/kg/day
Haloperidol 1.0 mg/kg/day
Haloperidol 2.0 mg/kg/day
5.0 sec
2.5 sec
1.0 sec
0.5 sec
Omissions
Anticipatory Responses
Perseverative Responses
*
#
#
#
#
*

Fig 4.5

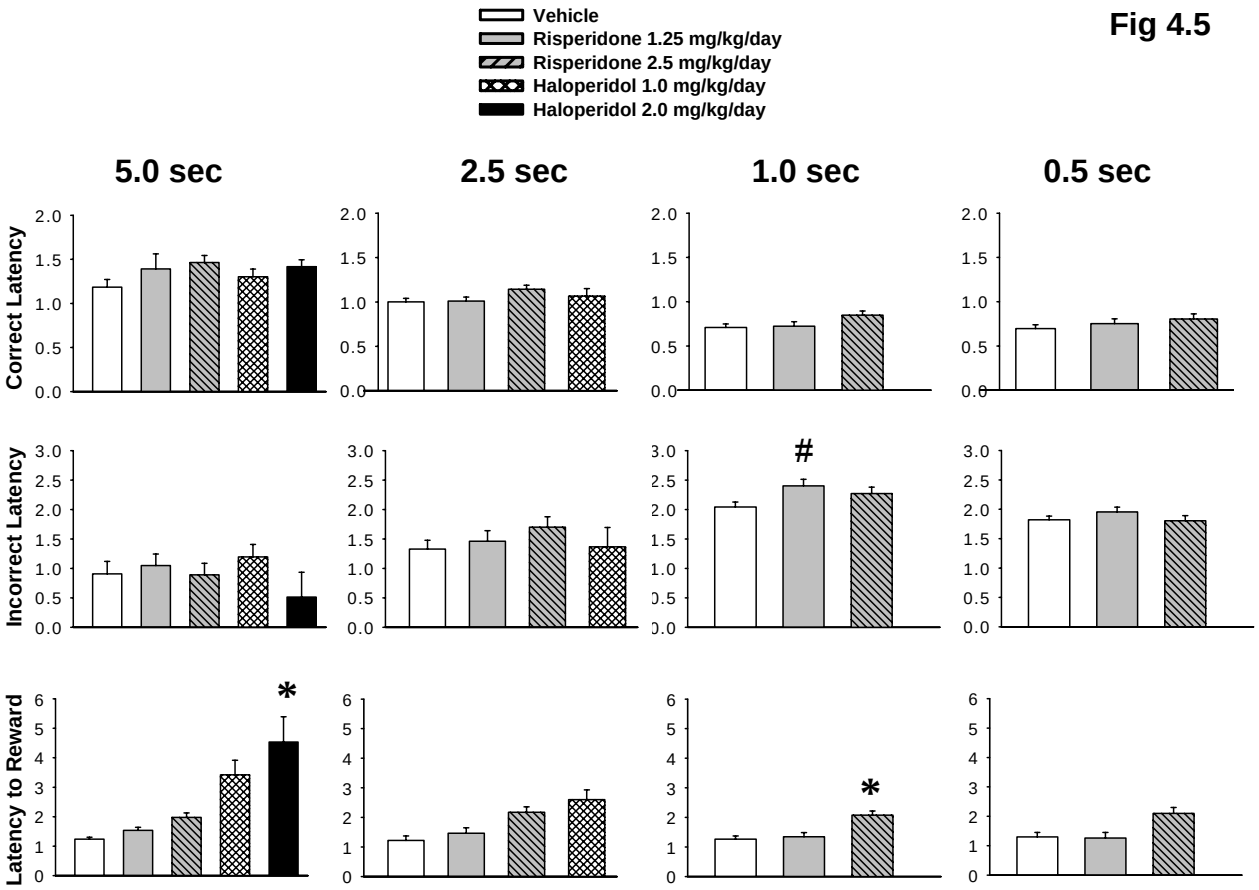
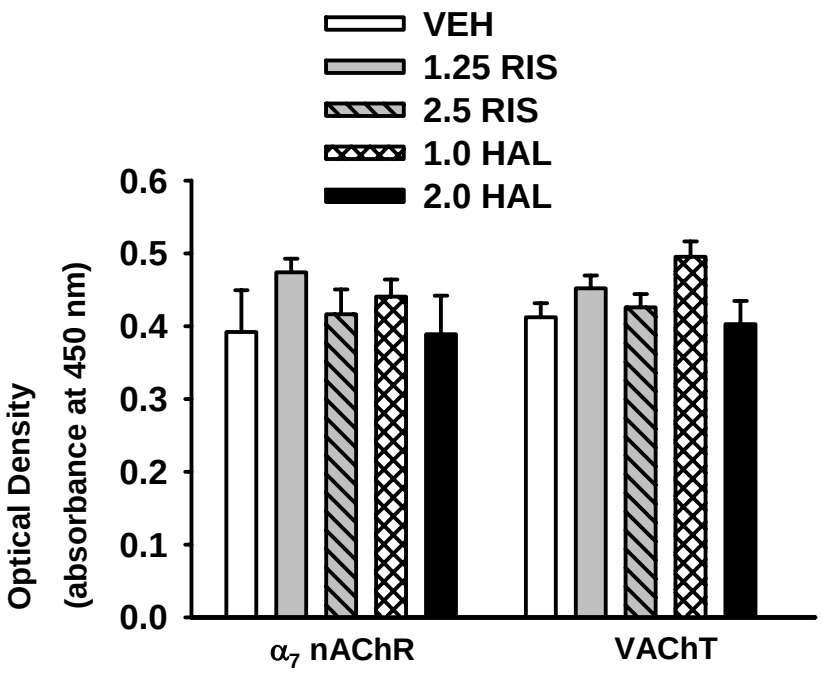

Vehicle
Risperidone 1.25 mg/kg/day
Risperidone 2.5 mg/kg/day
Haloperidol 1.0 mg/kg/day
Haloperidol 2.0 mg/kg/day
5.0 sec
2.5 sec
1.0 sec
0.5 sec
Correct Latency
Incorrect Latency
Latency to Reward
#
*
*

Fig 4.6


VEH
1.25 RIS
2.5 RIS
1.0 HAL
2.0 HAL
Optical Density
(absorbance at 450 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
α7 nAChR
VAChT

CHAPTER 5

CONCLUDING REMARKS

This dissertation addressed three specific aims. In the evaluation of two commonly used acetylcholinesterase inhibitors for effects on sensorimotor gating in an experimental animal model (specific aim 1), there were three main findings. First, under vehicle conditions, there were no significant effects of repeated exposures to the prepulse inhibition test method on startle amplitude or prepulse inhibition in rats for up to 3 sessions (i.e., after the initial test session to establish baselines). Second, neither galantamine nor donepezil had any significant effect on startle amplitude or prepulse inhibition on their own. Third, galantamine (depending on dose) improved prepulse inhibition deficits in all three pharmacologic models of prepulse inhibition impairment, whereas donepezil ameliorated prepulse inhibition deficits induced by scopolamine and apomorphine, but was not effective in the MK801 model. From these findings, we concluded that the clinically used acetylcholinesterase inhibitors, galantamine and donepezil, have the ability to improve auditory sensory gating in established pharmacologic models of impaired prepulse inhibition. These data combined with the positive clinical data suggest that efforts to increase cholinergic activity (i.e., by cholinesterase inhibitors or other means) in the brain warrant further investigation as potential therapeutic options for schizophrenia and other conditions where prepulse inhibition is disrupted.

In the evaluation of the effects of representatives of different classes of neuroleptic drugs on cognitive function in experimental animal models (specific aim 2), there were five major findings in the subchronic dosing experiments. First, in radial arm maze

experiments neither haloperidol nor risperidone affected win-shift acquisition although delay-non-match-to-position performance was modestly impaired at the longer delays by risperidone. Second, haloperidol, but not risperidone, impaired water maze hidden platform acquisition as well as probe trial performance. Third, risperidone was without significant effect in the subsequent extinction trials and due to impairments in the first probe trial, it was not possible to fully assess the effects of haloperidol on extinction. Fourth, the level of thigmotaxis was elevated and swim speeds were somewhat reduced in the haloperidol-treated animals in the water maze possibly indicative of psychomotor impairments and elevated levels of anxiety. Lastly, the possible influence of elevated levels of anxiety in haloperidol-treated animals on the performance of memory-related tasks was further supported by subsequent experiments using the light-dark box and elevated plus maze tests.

In 5-choice serial reaction time task conducted under long term dosing (from day 90 to day 320 of exposure), there were three major findings. First, in 5CSRTT experiments haloperidol and, to a lesser degree, risperidone, impaired task acquisition as indicated by the failure (or increase in the number of trials) to meet specific performance criteria. Second, haloperidol treated animals failed to fully acquire the task whereas risperidone-treated animals performed more similarly to vehicle animals, but were generally impaired as the task became more difficult. Third, at both the longer and shorter stimulus durations both antipsychotics generally increased the number of perseverative responses as well as latencies to respond to stimuli and collect rewards.

Results from these behavior experiments indicate task dependent and temporal effects of exposure to therapeutic doses of haloperidol and risperidone. Subchronic exposure

to haloperidol (but not risperidone) leads to impairments in the performance of a spatial reference learning procedure. These deficits may in part be due to haloperidol-related psychomotor impairments and elevated levels of anxiety. In addition, risperidone may impair spatial working and short-term memory as the demands of the task increase. Further, chronic exposure to therapeutic doses of haloperidol and risperidone (to a lesser degree) leads to impairments in the acquisition of a task of sustained attention, vigilance, and executive function. As in Morris water maze, haloperidol impairments in 5-CSRTT were apparent early in testing. Such impairments may in part be due to alterations in signal detection, psychomotor speed, and reward motivation. Similar to the radial arm maze experiments, risperidone, however, produced negative effects late in testing (i.e., during the more difficult sessions). Although in 5CSRTT, the cognitive factors involved are attention and executive function (as opposed to spatial and short term memory of radial arm maze), results indicated that risperidone-induced impairments may directly correlate with increasing demands of the task.

In the attempt to determine if a relationship exists between neuroleptic-induced effects on the central cholinergic system and the behavioral data (described above), we found that haloperidol and risperidone were without significant effect in the ELISA experiments which were conducted to detect potential (antipsychotic-related) alterations in the levels of vesicular acetylcholine transporter and α7 nicotinic acetylcholine receptor in medial prefrontal cortex of rats treated for 320 days. Thus, behavioral impairments associated with long term antipsychotic treatment, do not appear to be due to deficits in vesicular acetylcholine transporter or α7 nicotinic acetylcholine receptor in medial prefrontal cortex, but may instead be due to either alterations of other cholinergic proteins or the

modulation of other neurotransmitter systems.

Within this project, data supported my **first hypothesis** that enhancement of the cholinergic system by acetylcholinesterase inhibitors would improve cognitive function in an animal behavior procedure that models auditory gating deficits, one feature of schizophrenia. Data collected for this project also supported our **second hypothesis** that atypical neuroleptics when administered chronically would be superior to typical neuroleptics in animal models of sustained attention and working memory, but I found no evidence that this finding is due to less deleterious effects on the cholinergic system in the brain. However, further exploration of possible cholinergic deficits in other markers (muscarinic receptors, choline acetyl transferase, etc.), and by other methods (immunoblots, sterology, etc.), and in other brain regions is one future direction for work described in this dissertation. Such molecular testing is essential to truly establish whether or not atypical and typical antipsychotics affect the cholinergic system at 320 days of exposure. Due to the uniqueness of this length of drug exposure, we would also like to examine effects on dopamine and serotonin systems as vigorously as the amount of collected tissue permits.