Title Page

Differential chronic effects of haloperidol and risperidone on the acquisition and performance of tasks of spatial working/short term memory and sustained attention in rats

Elizabeth J. Hutchings, Jennifer L. Waller, and Alvin V. Terry, Jr.

Department of Pharmacology and Toxicology, Georgia Regents University, Augusta, Georgia, 30912 (EJH, AVT)

Department of Biostatistics, Georgia Regents University, Augusta, Georgia, 30912 (JLW)
Running Title Page

Running Title: Antipsychotics Impair Sustained Attention

Corresponding Author:

Alvin V. Terry Jr., Ph.D.
Department of Pharmacology & Toxicology
1120 15th Street, CB-3545
Georgia Regents University
Augusta, Georgia 30912
Phone: 706-721-9462
Fax: 706-721-2347
e-mail: aterry@gru.edu

Text format:

Number of text pages: 39
Tables: 2
Figures: 4
References: 36
Abstract: words 250
Introduction: words 741
Discussion: words 1596

Abbreviations:

HAL, haloperidol
5C-SRTT, five choice serial reaction time task
RISP, risperidone
SD, stimulus duration
Abstract:

A common feature of the neuropsychiatric disorders for which antipsychotic drugs are prescribed is cognitive dysfunction, yet the effects of chronic antipsychotic treatment on cognition are largely unknown. In the current study we evaluated the effects of chronic oral treatment with the first generation antipsychotic haloperidol (1.0 and 2.0 mg/kg/day), and the second generation antipsychotic risperidone (1.25 and 2.5 mg/kg/day) on the acquisition and performance of two radial arm maze (RAM) tasks and a five choice serial reaction time task (5C-SRTT), in rats during days 15-60 and 84-320 days of treatment, respectively. In the RAM neither antipsychotic significantly affected the acquisition or performance of a spatial win-shift or a delayed non-match-to-position task. Conversely, in the 5C-SRTT, haloperidol was associated with profound deficits in performance and the subjects were not able to progress through all stages of task acquisition. Depending on dose, risperidone was associated with a higher number of trials to meet specific performance criteria during task acquisition compared to vehicle-treated controls; however, most subjects were eventually able to achieve all levels of task acquisition. Both haloperidol and risperidone also increased the number of perseverative and timeout responses during certain stages of task acquisition and the response and reward latencies were slightly higher than controls during several stages of the study. These results in rats suggest that while chronic treatment with haloperidol or risperidone may not significantly affect spatial working/short-term memory, both antipsychotics can (depending on dose) impair sustained attention, decrease psychomotor speed, increase compulsive-type behaviors, and impair cognitive flexibility.
Introduction

The pharmaceuticals known as the “antipsychotics” rank among the most frequently prescribed medications in the United States and they are now used to treat pediatric patients (e.g., for pervasive developmental disorder, oppositional behavior, irritability and aggressive behaviors), young and mature adults (e.g., for schizophrenia, bipolar disease) and the elderly (e.g., for the behavioral and psychological symptoms of dementia). Such widespread prescribing is beginning to cause controversy given the paucity of controlled clinical trial data on antipsychotics given chronically, especially in young and elderly populations (see reviews, Seida et al., 2012; Huybrechts et al., 2012).

Another controversy relates to the selection of particular antipsychotics when their use is warranted. It had been widely held (since their advent in the 1980s), that the class of antipsychotics known as the “atypicals” or “second generation” antipsychotics (SGAs) were preferred over the older, “typical” or “first generation” antipsychotics (FGAs) primarily due to their lower incidence of extrapyramidal side effects. SGAs were also preferred for schizophrenia due to early reports of superior effects on negative symptoms, prevention of relapse, and increased functional capacity (see review, Miyamoto et al., 2005). The preference for SGAs over FGAs has been questioned recently, however, due to the emergence of serious long-term side effects of SGAs (e.g., abnormal weight gain, diabetes mellitus, hyperlipidemias etc.), cost concerns, and the results of large clinical trials including the Clinical Antipsychotic Trials in Interventions Effectiveness (CATIE) (Lieberman et al., 2005) and the Cost Utility of the Latest APDs in Schizophrenia Study (CUtLASS) (Jones et al., 2006) which suggested that FGAs and SGAs were essentially similar when overall efficacy or tolerability were compared.
A common feature of many of the conditions for which antipsychotics are prescribed is cognitive dysfunction, yet the effects of chronic antipsychotic treatment on cognition are unclear. Most of the available clinical data regarding antipsychotics and cognition were obtained from studies of adult patients with schizophrenia. As in the case of efficacy (i.e., for positive and negative symptoms of schizophrenia) and tolerability comparisons described above, early reports suggesting that SGAs improved cognition in schizophrenia (and were superior to FGAs in this regard) have not been confirmed in more recent studies where randomized double-blind treatment conditions were maintained, acceptable dosing comparisons between SGAs and FGAs were made, and potential practice effects in the cognitive tasks were considered (see Hill et al., 2010; Goldberg et al., 2010). Further, in the Clinical Antipsychotic Trials of Intervention Effectiveness–Alzheimer’s Disease study (CATIE-AD), SGAs (compared to placebo) were associated with greater rates of decline in cognitive function (Vigen et al., 2011).

When interpreting the available clinical data on the effects antipsychotics on cognition, one major limitation has been the relatively short duration of most of the studies. Antipsychotics are often prescribed for decades to patients with neuropsychiatric disorders, yet the clinical trial evaluations of these drugs rarely exceed several months to one or two years (presumably due to the costs and challenges associated with monitoring neuropsychiatric patients for extended periods). Accordingly, multi-year, prospective clinical studies designed specifically to identify antipsychotics that have optimal effects on cognition have not been conducted. Relevant animal studies may, therefore, be especially important in this context since they are more practical for long-term, prospective treatment evaluations. To date, only a small number of animal studies have been conducted where the chronic effects of antipsychotic treatment on cognition have been evaluated, however. These studies include experiments where seventy-five days of treatment...
with clozapine, haloperidol, and risperidone in drinking water impaired acquisition of an eight-arm radial maze in both young and aging rats while olanzapine had no effect (Rosengarten and Quartermain, 2002). In another rat study, twenty-eight days of treatment with risperidone or haloperidol delivered with osmotic minipumps impaired working memory in a cross maze task (Karl et al., 2006). In a rat study where olanzapine was administered subcutaneously for twenty-one days, water maze (spatial learning) impairments were observed (Didriksen et al., 2006). In previous rat studies in our laboratory, where longer periods of treatment were evaluated (i.e., 90-180 days in drinking water), water maze spatial learning deficits were associated with haloperidol, chlorpromazine, risperidone, olanzapine, and ziprasidone (see review, Terry and Mahadik, 2007; Terry et al., 2007a and 2007b; Terry et al., 2008). The purpose of the study described here was to compare the effects of chronic treatment with the FGA haloperidol and the SGA risperidone on both the acquisition and performance of tasks designed to assess spatial working/short term memory and sustained attention in rats.
Methods

Animal Care

All procedures employed during this study were reviewed and approved by the 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°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 daily for several minutes for four days prior to the initiation of behavioral testing.

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 (see Terry et al., 2007; Terry et al., 2010). Furthermore, for both haloperidol and risperidone, the doses selected (see below) would be expected to achieve comparable (and therapeutically) relevant D2 receptor occupancy values in vivo (i.e., in the range 65-80%; see (Kapur et al., 2003) based on the work of (Barth et al., 2006). Rats were thus treated with 1.0 or 2.0 mg/kg/day haloperidol (4-[4-(4-Chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one, Sigma-Aldrich, St. Louis, MO) or 1.25 or 2.5 mg/kg/day risperidone (4-[2-[4-(6-
fluorobenzo[d]isoxazol-3-yl)-1-piperidyl]ethyl]-3-methyl-2,6-diazabicyclo[4.4.0]deca-1,3-dien-5-one, 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.

**Behavioral testing**

Study subjects were transferred (in their home cages) to the behavioral testing rooms each morning approximately 30 min before the beginning of experiments.

**Radial Arm Maze Procedures**

Beginning on day 15 of antipsychotic treatment, radial arm maze testing (as we have described previously, see Terry et al., 2008) began using two Med-Associates (MED-RAM-1R) computer-automated, 8-Arm Radial Mazes consisting of a central octagonal hub (arena) with automatic guillotine doors connected to aluminum arms (8.9 cm wide) 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 mazes were positioned approximately 90 cm above the floor in a testing room with a number of extra-maze cues (composed of large geometrical shapes).

**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 (45 mg Dustless Precision Pellets, Bioserve, Frenchtown, NJ.) 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 (see below) if criterion was met. Criterion of win-shift was completion of the task within the time limit (15 minutes) on four consecutive days with \( \leq \) 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 \( \leq \) one error during the free eight sessions, animals advanced to longer delays.
of one, three, and six hours. Each longer delay (along with additional 15 min delay sessions) were randomly presented twice.

**Five Choice Serial Reaction Time Task (5C-SRTT)**

Training and testing in 5C-SRTT was initiated on day 84 of antipsychotic treatment and conducted using six ventilated, sound attenuated operant chambers (Med Associates, St. Albans, VT, USA) as we have described previously (Terry et al., 2012). Each operant chamber consisted of 9 nose pokes/apertures, 4 of which were closed off with metal inserts, leaving every other nose poke available (2.5cm wide, 4cm deep). The apertures were arranged on a curved panel 2 cm above the floor of the chamber and were equipped with a photocell beam to detect nose pokes. There was a lamp (2.8W) on the rear wall of each aperture that could be illuminated randomly and for varying durations. Food pellets (45 mg Dustless Precision Pellets, Bioserve, Frenchtown, NJ.) were delivered automatically to a magazine on the opposite wall to the nose pokes. A light inside the food magazine was also turned on to indicate that a pellet (45mg chow pellet, BioServ, Frenchtown, NJ, USA) had been dispensed. The food magazine was equidistant from all nose poke apertures. There was a house light that remained on for the entire session unless an error or omission occurred; the light was located towards the roof of the operant chamber above the magazine. The apparatus was controlled using MedPC software (Med Associates, St. Albans, VT, USA).

*Training and testing procedures*- During each session, the rat was 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 sec). 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 was rewarded with a food pellet, and each failure to respond (omissions, longer than 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) was punished with a 10 sec time-out with no access to a food pellet.

The following parameters were measured to assess performance: 

- $\% \text{ correct} = \left( \frac{\# \text{ correct}}{\# \text{ correct} + \# \text{ incorrect}} \right) \times 100$;
- $\% \text{ omissions} = \left( \frac{\# \text{ omissions}}{\# \text{ trials completed}} \right) \times 100$;
- $\# \text{ of premature responses (impulsivity)} = \# \text{ responses made after a trial began, but before onset of the light stimulus (i.e., during the 5 second intertrial interval)}$;
- $\# \text{ of perseverative responses (compulsivity)} = \# \text{ nose pokes made after the correct response was made (i.e., in same aperture), but before collecting the reward}$;
- $\# \text{ of timeout responses} = \# \text{ nose pokes made in any aperture during a timeout period}$;
- $\text{Latency to correct} = \text{time elapsed (sec) from the onset of the light stimulus to making the correct nose poke response}$;
- $\text{Latency to incorrect} = \text{time elapsed (sec) from the onset of the light stimulus to making the incorrect nose poke response}$;
- $\text{Latency to reward} = \text{time elapsed (sec) from making a correct nose poke response to retrieving the food reward from the magazine}$.

Animals (n=8-10 per treatment/vehicle group) moved through a series of five criterion points to complete the task. 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 sec stimulus length and 10 sec response time; 5 sec stimulus time/5 sec response time; 2.5 sec stimulus/5 sec response time; 1 sec stimulus/5 sec response time; and 0.5 sec stimulus/5 sec response time. Criterion was defined as five consecutive days at 90% or higher correct responses for the first three stimulus durations, but was decreased to 75% or higher for the last two stimulus durations. Animals were allowed a maximum of 150 days to reach and complete the final criterion phase.
an animal tested for 80 days at any one phase without progressing, it was discontinued from the task, and the sessions to criterion was recorded as 80.

Statistical Analyses

Statistical analysis was performed using SigmaPlot 11.2 and SAS 9.3 and statistical significance was assessed using an alpha level of 0.05. For all two factor comparisons (i.e., treatment, test session) in the RAM and 5C-SRTT studies, a mixed model analysis of variance (with repeated measures) was used followed by the Student Newman Keuls method for post hoc analysis. For the various stages of task acquisition in the 5C-SRTT, Chi-square or Fisher’s Exact tests (if the assumptions to the chi-square test were violated) were used to examine differences between treatment groups within stimulus duration of the proportion of subjects meeting criteria. To compare each treatment to vehicle, chi-square or Fisher’s Exact tests were used and an Bonferroni adjustment to the overall alpha level was used to control for the number of comparisons within stimulus level. To examine differences in the number of days (sessions) to meet criteria by treatment group and stimulus duration, a repeated measures mixed model was used and included main effects of treatment group, stimulus duration, and the two-factor interaction between treatment group and stimulus duration. Post hoc pair-wise comparisons were performed using a Bonferonni adjustment to the overall alpha level for the number of comparisons made.

Results

Chronic treatment with haloperidol or risperidone does not alter radial arm maze acquisition or performance.

Win-shift Acquisition
Fig 1A illustrates the effects of risperidone and haloperidol on the acquisition of a win-shift task in a computer-automated, eight-arm radial arm maze. There was no main effect of treatment on the number of errors committed (p=0.29) nor was there a significant treatment by session interaction (p=0.35). There was a significant effect of the day of testing (F(10,692)=35.16, p<0.001) indicating that the test subjects in all treatment groups improved as they completed more test sessions. There were also no significant treatment-related differences in the number of sessions required to reach criterion (p=0.72), see Fig 1A inset.

Delayed Nonmatch-to-Position (DNMTP)

The results of the DNMTP (also referred to as a delayed spatial win-shift task, see Taylor et al., 2003) training at 15-min delays are presented in Fig 1B. There were no significant treatment-related differences in the number of sessions to reach criterion (p=0.80). Likewise, there were no significant differences in the number of errors committed (i.e., combination of working and reference memory errors, p=0.50, see below), nor was there a significant treatment by day interaction on errors made across groups (p=0.26, data not shown). With the introduction of longer delays (Figure 1C), the number of errors increased in all treatment groups (F(3,224)=32.53, p<0.001), however, there were no significant treatment-related differences (p=0.18) nor was there a significant treatment by delay interaction (p=0.63). It should be noted, that in this portion of the study the clear majority of the errors made were reference memory errors (entries into unbaited arms that were active during the previous information session). Working memory (reentries into an arm that had previously delivered a reward during the retention session) and reference memory errors were analyzed separately as well as combined (total errors as illustrated in the figures). The same results (no significant treatment related differences) were observed.
Chronic treatment with haloperidol or risperidone is associated with alterations in the acquisition and performance of a Five Choice Serial Reaction Time Task (5C-SRTT).

Achievement of Training Criteria

The effects of chronic treatment with haloperidol and risperidone on the proportion of subjects meeting the predefined performance criteria (see Methods) and the number of test sessions required to meet criteria at each stimulus duration in a 5C-SRTT are illustrated in Table 1A and 1B, respectively. In Table 1A, * indicates a lower proportion of animals reaching criteria compared to vehicle-treated controls within stimulus duration (descriptive statistics by treatment group and stimulus duration, p<0.0125, Bonferroni adjusted post hoc alpha level). For the number of sessions required to reach criterion, a repeated measures mixed model indicated a highly significant main effect of treatment, \( F_{(4,13)} = 16.57, p<0.0001, \) a significant effect of the stimulus duration \( F_{(4,9)}=6.53, p=0.01, \) with a non-significant treatment x stimulus duration interaction \( (F_{(12,9)}=1.21, p=0.40). \) Post hoc analysis indicated that within the 10 sec stimulus duration, both haloperidol treatment groups required a higher mean number of days (sessions) to achieve criteria \( (p=0.002 \) and \( p<0.0001 \) for haloperidol 1.0 and 2.0, respectively). At the 5-sec stimulus duration, both doses of haloperidol and the higher dose of risperidone were associated with a higher mean number of days to achieve criteria \( (p=0.0001, p<0.0001, \) and \( p=0.003 \) for haloperidol 1.0, 2.0, and risperidone 2.5 mg/kg/day, respectively).

For each of the 5C-SRTT performance measures described below, the results of first 10 individual days of training are provided (to illustrate the initial acquisition of the task) followed by the mean of the first 5 days of testing at each of the lower stimulus durations (SDs).

Accuracy (% correct)
The effects of chronic treatment with haloperidol and risperidone on accuracy across the various stimulus durations are illustrated Fig 2 (top row, left column) and Fig 3 (top row). While performance in the subjects treated with the higher dose of haloperidol (2.0 mg/kg/day) was slightly inferior to control subjects at the 10 sec stimulus duration, the percentage of correct nose pokes increased progressively in all treatment groups over the course of the first 10 sessions of training. As indicated in Table 1, however, the number of test sessions required to reach criteria (five consecutive sessions at 90% correct or higher) was clearly higher in both haloperidol treatment groups compared to vehicle controls and (in fact) only two of the eight subjects administered the 2.0 mg/kg/day dose actually met criteria. Therefore, rats administered the higher dose of haloperidol were not able to progress past this stimulus duration. At the 5.0 sec stimulus duration, the following observations were made: Accuracy was impaired in all of the remaining antipsychotic treatment groups during the first 5 test sessions, and the number of trials required to achieve criteria (five consecutive sessions at 90% correct or higher) was significantly higher in all treatment groups compared to controls) except those administered risperidone 1.25 mg/kg/day. Accuracy was also slightly (but significantly) impaired in subjects administered the higher (2.0 mg/kg/day) dose of risperidone at the 2.5 and 1.0 sec stimulus durations. Note: haloperidol 2.0mg/kg/day is not illustrated at the 5 sec stimulus duration since the subjects did not progress to this level. Likewise, most subjects administered the 1.0 dose of haloperidol did not progress past the 5 sec stimulus duration and thus this dose is not depicted in the figures where shorter stimulus durations are presented.

Premature Responses

The effects of chronic treatment with haloperidol and risperidone on the number of premature responses across the various stimulus durations are illustrated in Fig 2 (middle row,
left column) and Fig 3 (middle row). There were no statistically significant differences in premature responses noted across the treatment groups.

**Perseverative Responses**

Antipsychotic effects on the number of perseverative responses across the various stimulus durations are illustrated in Fig 2 (middle row, center column) and Fig 3 (bottom row). As illustrated, during the first ten sessions of training, both doses of haloperidol were associated with a significantly higher number of perseverative responses compared with vehicle controls. This effect of haloperidol was also present at the 5 sec stimulus duration with the 1.0 mg/kg/day dose. Risperidone (either the 2.5 or both the 2.5 and 1.25 mg/kg/day doses) was also associated with a higher number of perseverative responses at the 5.0, 2.5. and 1.0 sec stimulus durations.

**Timeout Responses**

The effects of the antipsychotics on the number of timeout responses across the various stimulus durations are illustrated in Fig 2 (middle row, right column) and Fig 4 (top row). At the beginning of 5C-SRTT training (at the 10 sec stimulus duration) the number of timeout responses began relatively high (40-60), was not statistically different between the treatment groups, and progressively decreased over the course of the first 10 sessions. At the five sec stimulus duration, the number of timeout responses was higher (compared to vehicle controls) in the subjects administered haloperidol 1.0 mg/kg/day as well as those administered risperidone 1.25 or 2.5 mg/kg/day. In addition, both doses of risperidone were associated with increases in timeout responses at the 1.0 sec stimulus duration and the 2.5 mg/kg/day dose of risperidone was also associated with increases in timeout responses at the 0.5 sec stimulus duration.

**Response Latencies**
The response latencies associated with correct and incorrect responses and with collecting food rewards across the various stimulus durations are illustrated in Fig 2 (bottom row) and Table 2. At the beginning of 5C-SRTT training (at the 10 sec stimulus duration) the latencies (for both correct and incorrect responses) began around 3.5-5.0 sec and progressively decreased over the course of the first 10 sessions. For correct choices, response latencies were slightly elevated in all antipsychotic groups (compared to vehicle controls) at the 10.0, 5.0, and 2.5 sec stimulus durations. At the lower (1.0 and 0.5 sec) stimulus durations, where only risperidone-treated subjects remained, no significant differences from control were noted. For incorrect choices, only the 2.0 mg/kg/day dose of haloperidol (at the 10 sec stimulus duration) was associated with higher response latencies. At the beginning of 5C-SRTT training (at the 10 sec stimulus duration) the reward latencies began around 6.0-10.0 sec and progressively decreased over the course of the first 10 sessions. As illustrated, both doses of haloperidol and the higher dose of risperidone were associated with slightly higher latencies to collect food rewards (compared to vehicle controls) at all stimulus durations where treatment effects were compared.

Omissions

The percentage of omissions across the various stimulus durations are illustrated in Fig 2 (top row, center column) and Fig 4 (bottom row). At the beginning of 5C-SRTT training (at the 10 sec stimulus duration) omissions in all treatment groups were relatively high (~ 60), but progressively decreased over the course of the first 10 sessions. Statistical comparisons indicated the following: that omissions were higher (compared to control) in subjects administered haloperidol 2.0 mg/kg/day at the 10 sec stimulus duration, haloperidol 2.0 mg/kg/day and both doses of risperidone at the 5.0 sec stimulus duration, and both doses of risperidone at the 2.5 sec stimulus duration.
Discussion

The results of this study can be summarized as follows: 1) in the RAM neither antipsychotic significantly affected the acquisition or performance of a win-shift or DNMT task, 2) in the 5C-SRTT, haloperidol was associated with profound deficits in performance and none of the test subjects were able to progress through all stages of task acquisition, 3) risperidone was associated with a higher number of trials to meet specific performance criteria during task acquisition compared to controls; however, most subjects were able to achieve all levels of task acquisition. 4) Both haloperidol and risperidone increased the number of perseverative and timeout responses during certain stages of task acquisition and the response and reward latencies were slightly higher than controls in several phases of the study.

The first observation (lack antipsychotic effects in the RAM) was somewhat surprising, given the aforementioned study where 75 days of treatment with haloperidol and risperidone in drinking water impaired RAM acquisition in both young and aging rats (Rosengarten and Quartermain, 2002). In our study, the length of previous drug exposure was shorter (testing began after 15 days of treatment versus 75 days) and there were some differences in the test apparatus between the studies that could have resulted in differences in task difficulty. For example, the arm widths and lengths were 12 and 60 cm, respectively, in the Rosengarten and Quartermain study versus 8.9 and 45.7 cm, respectively, in our study. Other possible sources of the disparate results could include differences in intra-maze and extra-maze cues, and differences in the strain of rats tested (Fisher 344 in the Rosengarten and Quartermain study versus Wistar rats in our study). It is important to note, however, that in a previous study in our laboratory using a 12-arm RAM with arms 10 cm wide and 70 cm long (with testing begun during washout after a 90 day drug exposure period in Wistar rats), neither haloperidol nor risperidone affected
the number of working or reference memory errors compared to vehicle-treated controls (Terry et al., 2007b).

In the second phase of this study, we were interested in the effects of chronic antipsychotic treatment on the acquisition and performance of a task of sustained attention in rats. Among the variety of cognitive deficits reported in schizophrenia patients (who are often administered antipsychotics chronically), impairments in sustained attention, as assessed by the continuous performance test (CPT, Rosvold et al., 1956), are a relatively consistent observation (Nuechterlein and Dawson, 1984; Cornblatt and Keilp, 1994) and they often persist during both active psychotic episodes and periods of remission. Hence, we employed the rodent 5C-SRTT (Robbins 2002) which is thought to model the human CPT. In the 5C-SRTT, attention and inhibitory response control (thought to be a form of executive functioning) is indexed by the accuracy measurement. Our first notable observation was that haloperidol markedly impaired performance of the 5C-SRTT so that subjects were not able to progress through many stages of task acquisition. Risperidone was also associated with a higher number of trials to meet specific (accuracy-related) performance criteria, however, (as noted above) most subjects eventually achieved all levels of task acquisition suggesting that risperidone may be superior to haloperidol when sustained attention is concerned.

Another notable observation in the 5C-SRTT experiments was that both antipsychotics increased perseverative responses (i.e., a repeated nose poke into the same aperture after a correct response) as well as timeout responses (i.e., nose pokes made during the timeout interval which occurred after an incorrect response or an omission) during certain stages of task acquisition. Perseverative responses in the 5C-SRTT are generally interpreted as a form of compulsive-like behavior. Timeout responses have been suggested to represent both a form of
compulsive-like behavior and/or cognitive inflexibility (i.e., the inability to alter behavior in reaction to changing situational demands, in this case, disorganized responses that are not tied to the stimulus presentation, see Amitai and Markou, 2010). These observations in animals suggest that cognitive inflexibility which is likely innate in many patients suffering from schizophrenia and other mental health disorders (see Amitai and Markou, 2010) could be exacerbated by chronic antipsychotic exposure.

The modest increases in response latencies, reward latencies, and omissions in several portions of the study (i.e., observed in all antipsychotic-treated groups depending on the dose and the phase of task acquisition), suggest that the drug effects on 5C-SRTT acquisition (which was primarily based on accuracy-related performance criteria) may in part have been due to alterations in signal detection, psychomotor speed, or more overt effects on motor function or reward motivation. To argue against the later possibility, it is important to note that omissions were relatively low (less than 15% for most phases of the study) and the reward latencies typically were well below 3 sec for most phases of the study.

Our results with haloperidol in the 5C-SRTT are in general agreement with another study in rats where the FGA was administered for 3 months (i.e., injected daily 45 min before behavioral testing) and found to disrupt sustained attention and to increase response latencies as well as the number of omissions (Brockel and Fowler 1995). Interestingly, in a task designed to maximize attention and minimize movement requirements, haloperidol (administered acutely) increased errors of omission and reaction time, however the lack of a significant correlation between these two measures suggested that attentional accuracy may be independent of the motor slowing effect (Skjoldager and Fowler, 1991). A more recent study found that both haloperidol and risperidone (administered acutely) impaired sustained attention in an operant
visual signal detection task and that both antipsychotics increased response latencies, while haloperidol (but not risperidone) increased the rate of response omissions (Rezvani and Levin, 2004).

The 5C-SRTT studies suggest that chronic exposure to either haloperidol or risperidone may lead to performance deficits of tasks that require sustained attention especially those that require rapid response times and motor actions (i.e., deficits that could have important implications for a number of day to day activities in psychiatric patients). Notably, concerns have been raised about the higher rate of automobile accidents (and the potential involvement of antipsychotics) in psychiatric patients who often demonstrate psychomotor impairment (see Brunnauer et al., 2004).

Collectively, results of the animal experiments described here may be especially important given the limitations of the available clinical studies which often were relatively short in duration, conducted in small numbers of patients, and not placebo controlled (especially in schizophrenia), thus making it difficult to distinguish drug effects from disease symptoms. Moreover, the clinical studies have often yielded equivocal results. For example, one older study found that chronic haloperidol impaired spatial working memory in schizophrenic smokers (Levin et al., 1996), however, the test subjects were deprived of cigarettes at the time of cognitive testing introducing the potential confound of the withdrawal effects of nicotine. In a more recent study, risperidone was found to improve spatial working memory compared to haloperidol, however, this superiority was eliminated when the confounding effects of the anticholinergic benzotropine (administered adjunctively with haloperidol) were considered (McGurk et al., 2004). Other studies in schizophrenia have documented both improvements (McGurk et al., 2005) and impairments (Reilly et al., 2006) in spatial working memory.
associated with chronic risperidone treatment. The clinical effects of antipsychotics on sustained attention also appear to be equivocal. For example, while both a 12-week (Liu et al., 2000) and 2-year (Green et al., 2002) comparison of haloperidol and risperidone on performance of a CPT found no significant drug-related effects, Chen et al., 2004 reported plasma concentration dependent impairments in the CPT associated with risperidone and its active metabolite 9-hydroxy-risperidone after 1 year of exposure.

There are some limitations of the current study that should be discussed. As noted in the Methods, the dosing approach in drinking water was based on previous studies where plasma antipsychotic levels fit in the range associated with therapeutic responses in schizophrenia patients. However, only the 2.0 mg/kg and 2.5 mg/kg doses haloperidol and risperidone, respectively, were evaluated and it is unclear if the lower doses (1.0 mg/kg and 1.25 mg/kg) behaviorally tested here would generate therapeutic plasma levels. Further, the doses used in very young and elderly patients are generally lower than those used in adult schizophrenics, thus it is unclear if the behavioral effects described here would be relevant for these patient populations. Finally, this study was conducted in normal Wistar rats, thus it is unclear if similar antipsychotic effects would be observed in neuropsychiatric disease-related animal models. The differential sensitivity to the antipsychotics in the RAM versus the 5C-SRTT may at least in part be due to the longer exposure times in the 5C-SRTT. While (as noted above) we have evaluated antipsychotic exposure times up to 90 days in the RAM, we have not evaluated exposure times as long as evaluated in the 5C-SRTT in this study (i.e. 84-320 days). An additional possibility for the differential drug sensitivity is the nature of the behavioral tasks (i.e., free exploration and foraging behaviors in the RAM versus nose-poke (operant-based) behaviors in 5C-SRTT where the environment is more restrictive). It is thus interesting to speculate that the more natural
(ethologically relevant) RAM may simply be less difficult to master and therefore less sensitive to drug impairment.

In conclusion, the results of this study in rats suggest that while chronic treatment with haloperidol or risperidone may not significantly affect spatial working/short-term memory, both antipsychotics can impair sustained attention (in a dose-dependent manner), decrease psychomotor speed, increase compulsive-type behaviors, and impair cognitive flexibility. Given the widespread use of these agents across multiple neuropsychiatric disorders and patient populations, such long term effects observed in animals should be considered.
Acknowledgements

The authors would like to thank Ms. Ashley Davis for her administrative assistance in preparing this article.
Authorship Contribution

Participated in research design: Hutchings, EJ, Terry, A.V., Jr.

Conducted experiments: Hutchings, EJ

Performed data analysis: Hutchings, EJ, Waller, JL, Terry, A.V., Jr.

Wrote or contributed to the writing of the manuscript: Hutchings, EJ, Waller, JL, Terry, A.V., Jr.
References

Amitai N, Markou A (2010) Disruption of performance in the five-choice serial reaction time task induced by administration of N-methyl-D-aspartate receptor antagonists: relevance to cognitive dysfunction in schizophrenia. Biol Psychiatry 68(1):5-16.


Footnotes:

The work described in this manuscript 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].
Legends for Figures

**Fig 1.** Antipsychotic effects on radial arm maze performance conducted during days 15-60 of treatment. **A.** Win-shift acquisition over 10 consecutive days of testing as assessed by the number of errors per session (mean ± S.E.M.). **Inset.** Win-shift acquisition as assessed by the number of trials to reach a pre-determined criterion (mean ± S.E.M.) which was defined as four consecutive sessions with ≤ 2 total errors.  **B.** Acquisition of a delayed non match to position (DNMTP) task at 15 min delays as assessed by the number of trials to criterion (mean ± S.E.M.) which was defined as ≤ 1 error for four consecutive sessions during the free eight sessions.  **C.** Delay dependent performance of the DNMTP task as assessed by the number errors per session (mean ± S.E.M.).

**Fig 2.** Antipsychotic effects on the initial acquisition of a five-choice serial reaction time task conducted during days 84-320 of treatment. Rats were treated with vehicle (VEH), haloperidol (HAL), or risperidone (RISP) in their drinking water (doses listed are mg/kg/24 hr) and then trained to meet specific performance criteria (explained in the text). The individual graphs show the first 10 sessions of training at the 10 sec stimulus duration (SD). Each symbol represents the mean ± SEM for each test group. The following performance measures are illustrated: accuracy (% Correct); percentage of omissions (% Omissions); premature responses (Prem Resp); perseverative responses (Persev Resp); timeout responses (Timeout Resp); response latencies associated with correct choices (Latency-C); incorrect choices (Latency-I); and with nose pokes into the food hopper to obtain rewards (Latency-RW). Abbreviated antipsychotics with a dose and an asterisk (e.g., HAL 2.0*) included in the figure indicate a significant difference (p<0.05)
between the antipsychotic-dose represented and the vehicle-associated performance level (main effect across sessions). The statistical test used was repeated measures AVOVA.

**Fig 3.** Antipsychotic effects on the acquisition of a five-choice serial reaction time task conducted during days 84-320 of treatment as stimulus durations (SDs) decreased. Rats were treated with vehicle (VEH), haloperidol (HAL), or risperidone (RISP) in their drinking water (doses listed are mg/kg/24 hr) and then trained to meet specific performance criteria (explained in the text). The individual histograms show the mean ± SEM of the first 5 sessions of training at each SD that followed the initial (10 sec SD) training sessions (i.e., 5 sec down to 0.5 sec, see top of Fig). The following performance measures are illustrated: accuracy (% Correct); premature responses (Prem Resp); and perseverative responses. * = p<0.05 indicates a significant difference between the antipsychotic-dose represented and the vehicle-associated performance level (main effect across sessions). The statistical test used was repeated measures AVOVA. Note: For the Haloperidol treatment groups, only the 1.0 mg/kg dose is shown for the 5 sec stimulus duration since the remaining subjects failed to meet the training criteria (see methods) at the 5 sec or shorter SDs and therefore, did not progress past this level.

**Fig 4.** Antipsychotic effects on the acquisition of a five-choice serial reaction time task conducted during days 84-320 of treatment as stimulus durations (SDs) decreased. Rats were treated with vehicle (VEH), haloperidol (HAL), or risperidone (RISP) in their drinking water (doses listed are mg/kg/24 hr) and then trained to meet specific performance criteria (explained in the text). The individual histograms show the mean ± SEM of the first 5 sessions of training at each SD that followed the initial (10 sec SD) training sessions (i.e., 5 sec down to 0.5 sec, see
top of Fig). The following performance measures are illustrated: timeout responses (Timeout Resp); percentage of omissions (% Omissions). * = p<0.05 indicates a significant difference between the antipsychotic-dose represented and the vehicle-associated performance level (main effect across sessions). The statistical test used was repeated measures AVOVA. Note: For the Haloperidol treatment groups, only the 1.0 mg/kg dose is shown for the 5 sec stimulus duration since the remaining subjects failed to meet the training criteria (see methods) at the 5 sec or shorter SDs and therefore, did not progress past this level.
Table 1: Five Choice Serial Reaction Time Task (5C-SRTT) Acquisition Data

A: Proportion of Test Subjects Meeting Criteria

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N</th>
<th>10.0</th>
<th>5.0</th>
<th>2.5</th>
<th>1.0</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.60</td>
</tr>
<tr>
<td>Haloperidol 1.0 mg/kg/day</td>
<td>9</td>
<td>0.89</td>
<td>0.33*</td>
<td>0.22*</td>
<td>0.11*</td>
<td>na</td>
</tr>
<tr>
<td>Haloperidol 2.0 mg/kg/day</td>
<td>8</td>
<td>0.25*</td>
<td>0.00*</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Risperidone 1.25 mg/kg/day</td>
<td>8</td>
<td>1.00</td>
<td>0.88</td>
<td>0.75</td>
<td>0.75</td>
<td>0.50</td>
</tr>
<tr>
<td>Risperidone 2.5 mg/kg/day</td>
<td>8</td>
<td>1.00</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
<td>0.38</td>
</tr>
</tbody>
</table>

B: Number of Test Sessions Required to Reach Criteria, Mean (SD)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N</th>
<th>10.0</th>
<th>5.0</th>
<th>2.5</th>
<th>1.0</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>13.10 (3.70)</td>
<td>7.20 (1.87)</td>
<td>11.60 (8.02)</td>
<td>10.80 (11.48)</td>
<td>40.60 (28.73)</td>
</tr>
<tr>
<td>Haloperidol 1.0 mg/kg/day</td>
<td>9</td>
<td>39.11 (23.87)**</td>
<td>60.00 (28.49)**</td>
<td>30.67 (43.14)</td>
<td>45.50 (48.79)</td>
<td>na</td>
</tr>
<tr>
<td>Haloperidol 2.0 mg/kg/day</td>
<td>8</td>
<td>64.50 (28.72)**</td>
<td>80.00 (0.00)**</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Risperidone 1.25 mg/kg/day</td>
<td>8</td>
<td>14.25 (5.34)</td>
<td>32.88 (29.19)</td>
<td>32.14 (26.60)</td>
<td>8.50 (4.28)</td>
<td>26.67 (11.72)</td>
</tr>
<tr>
<td>Risperidone 2.5 mg/kg/day</td>
<td>8</td>
<td>15.75 (9.15)</td>
<td>49.00 (29.66)**</td>
<td>27.67 (11.20)</td>
<td>15.67 (6.65)</td>
<td>41.33 (27.29)</td>
</tr>
</tbody>
</table>

*p<0.0125; **p<0.01; ***p<0.001, significantly different than vehicle-treated control performance. na = not applicable since test subjects at this antipsychotic dose did not advance from the previous stimulus duration. SD = standard deviation.
Table 2. Effects of Haloperidol and Risperidone on Response Latencies at different stimulus durations in the Five Choice Serial Reaction Time Task

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Latency Type</th>
<th>Stimulus Duration (sec)</th>
<th>5.0</th>
<th>2.5</th>
<th>1.0</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correct Response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td>1.10±0.06</td>
<td>0.98±0.03</td>
<td>0.70±0.02</td>
<td>0.68±0.03</td>
</tr>
<tr>
<td>Risperidone 1.25 mg/kg</td>
<td></td>
<td></td>
<td>1.41±0.07*</td>
<td>1.14±0.04*</td>
<td>0.72±0.05</td>
<td>0.77±0.06</td>
</tr>
<tr>
<td>Risperidone 2.5 mg/kg</td>
<td></td>
<td></td>
<td>1.50±0.04*</td>
<td>1.16±0.04*</td>
<td>0.86±0.07</td>
<td>0.76±0.07</td>
</tr>
<tr>
<td>Haloperidol 1.0 mg/kg</td>
<td></td>
<td></td>
<td>1.48±0.09*</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>Incorrect Response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td>0.71±0.04</td>
<td>1.03±0.16</td>
<td>2.19±0.0.12</td>
<td>1.96±0.09</td>
</tr>
<tr>
<td>Risperidone 1.25 mg/kg</td>
<td></td>
<td></td>
<td>1.00±0.04</td>
<td>1.50±0.27</td>
<td>2.43±0.10</td>
<td>2.21±0.10</td>
</tr>
<tr>
<td>Risperidone 2.5 mg/kg</td>
<td></td>
<td></td>
<td>0.90±0.08</td>
<td>1.63±0.26</td>
<td>2.37±0.14</td>
<td>2.07±0.18</td>
</tr>
<tr>
<td>Haloperidol 1.0 mg/kg</td>
<td></td>
<td></td>
<td>0.92±0.07</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>Reward</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td>1.23±0.06</td>
<td>1.24±0.08</td>
<td>1.22±0.07</td>
<td>1.20±0.09</td>
</tr>
<tr>
<td>Risperidone 1.25 mg/kg</td>
<td></td>
<td></td>
<td>1.45±0.10</td>
<td>1.47±0.16</td>
<td>1.28±0.08</td>
<td>1.24±0.08</td>
</tr>
<tr>
<td>Treatment</td>
<td>Mean ± SEM</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Risperidone 2.5 mg/kg</td>
<td>1.92±0.22*</td>
<td>2.21±0.17</td>
<td>2.04±0.22*</td>
<td>1.92±0.27*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haloperidol 1.0 mg/kg</td>
<td>2.98±0.38*</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SEM. * = p<0.05; significant difference from Vehicle-treated group; . na = not applicable since test subjects at this antipsychotic dose did not advance from the previous stimulus duration.