THE EFFECTS OF SCOPOLAMINE ON
RAT SERIAL PATTERN LEARNING AND REVERSAL LEARNING

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The Effects of Scopolamine on Rat Serial Pattern Learning and Reversal Learning

Central cholinergic systems have been implicated in a variety of cognitive tasks including attention (Gallagher & Colombo, 1995; Hodges, Linder, Hogan, Jones, & Markus, 2009; Jones, Barnes, Kirkby, & Higgins, 1995; Klinkenberg & Blokland, 2010; Muir, Dunnett, Robbins, & Everitt, 1992; Muir, Robbins, & Everitt, 1992; Muir, Everitt, & Robbins, 1995; Robbins, Everitt, Marston, & Wilkinson, 1989), short-term spatial memory (Costa & Xavier, 2007; Hodges et al., 2009; Morris, Garrud, Rawlins, & O'Keefe, 1982), the utilization of response rules (Marston, Everitt, & Robbins, 1993; Marston, West, Wilkinson, Everitt, & Robbins, 1994), and memory for paired-associate memory tasks (Atri et al., 2004; DeRosa & Hasselmo, 2000; Saar, Grossman, & Barkai, 2001). The receptors for the cholinergic system are divided into two main types, muscarinic and nicotinic, and evidence has indicated differential roles of each with respect to the aforementioned cognitive tasks (Everitt & Robbins, 1997). Numerous studies have provided evidence that muscarinic antagonists, particularly those that block the M1 receptor subtype, result in the greatest deficits in learning and memory tasks (Ellis et al., 2006; Everitt & Robbins, 1997). In contrast, nicotinic antagonists do not disrupt learning and memory in the same way or to the extent as that observed with muscarinic antagonists (Ellis et al., 2006; Everitt & Robbins, 1997).

Another type of learning and memory shown to recruit muscarinic cholinergic processes is the acquisition and retention of sequential tasks (Chenoweth, 2007; Higgins,
Woodward, & Henningfield, 1989; Hodges et al., 2009; Klinkenberg & Blokland, 2010; Sitaram, Weingartner, & Gillin, 1978). These tasks are among the more complex tasks involved in everyday life, as they are implicated in behaviors such as planning and organizing (Fountain, 2006; Higgins et al., 1989). Furthermore, the impaired ability to engage in sequential tasks is symptomatic of numerous degenerative diseases, specifically those that are characterized by deficits in the cholinergic systems, such as Alzheimer’s disease and other dementias (Higgins et al., 1989; Hodges et al., 2009; Klinkenberg & Blokland, 2010). This has stimulated much interest in the field to find the underlying mechanisms involved in performance of such tasks to gain a greater understanding and potentially develop better diagnostic and treatment options for patients suffering from these debilitating illnesses.

With regards to sequential learning, research from our lab has found that the nicotinic antagonist mecamylamine only caused subtle impairments in the retention of a sequential task whereas scopolamine, a muscarinic antagonist, caused a severe disruption in memory (Chenoweth & Fountain, 2006). It should also be noted that in this exploration of a nicotinic versus muscarinic antagonist that the dose of mecamylamine (10 mg/kg) was considered high where as the dose for scopolamine (0.6 mg/kg) was considered only moderately high within the literature (Klinkenberg & Blokland, 2010). This evidence suggests that the nicotinic cholinergic system may only play a supporting role in the maintenance of sequential responses whereas the muscarinic cholinergic system is necessary for acquiring and maintaining correct responses in a sequential pattern.
Because of this evidence implicating the essential role of the muscarinic cholinergic system in sequential learning tasks, the present study focused on this receptor system.

**The Effects of Muscarinic Cholinergic Blockade in Sequential Learning Paradigms**

Numerous human studies have demonstrated these deficits by examining the effects of the muscarinic cholinergic antagonists atropine and scopolamine on sequential learning tasks. Three such studies have demonstrated that atropine impairs repeated acquisition of response sequences (Higgins et al., 1989), scopolamine impairs acquisition of a categorized serial learning task (Sitaram et al., 1978), and scopolamine also impairs working memory in a maze learning test (Thomas et al., 2008).

Higgins et al. (1989) trained human subjects on different 10-item sequences immediately before an injection of atropine, and then 30 minutes, 1.5, 3, 5, 7, 9, and 24 hours following administration of the drug. Subjects were allowed 20 trials to learn each new sequence within each of the eight separate sessions. Each sequence required subjects to depress keys “1”, “2”, or “3” on a keypad in a predetermined pseudo-random order. When subjects were trained in a “performance” condition, the 10-item sequence remained the same throughout the eight sessions. When in an “acquisition” condition, subjects learned a new sequence in each session. Subjects were cued as to whether they were in an acquisition or a performance phase, as both phases alternated within each of the eight sessions. Also, by assessing both phases within each session, a comparison of acquisition and retrieval within subjects could be made. Results showed that subjects were more sensitive to the effects of atropine in the acquisition phase as compared to the
performance phase. In both phases, atropine caused greater impairments than placebo in both overall error rates and overall response rates; however, a higher dose (6.0 mg) was needed to observe impairments in the performance phase than in the acquisition phase (3.0 mg). Additionally, this effect appeared to be greatest during the 1.5 – 5 hour range post drug administration (Higgins et al., 1989). This higher dose may indicate differing thresholds for each phase type. Additionally, the authors suggest that the acquisition phase may be under a greater level of stimulus control than the performance phase, thus making it more sensitive to changes in the availability of cholinergic processes. These data suggest that muscarinic cholinergic processes are necessary for acquiring and retaining sequences.

Sitaram et al. (1978) found similar impairments in acquisition in human subjects with scopolamine, a muscarinic antagonist. In this task, subjects learned a fixed sequence of 10 words within a category (e.g., fruits) following an injection of either scopolamine or methscopolamine. Subjects given scopolamine required more trials to reach criterion (recalling the 10 words in the correct sequential order in two consecutive trials). This effect was significantly reversed when subjects were then given arecholine, a cholinergic agonist. These results also support the idea that muscarinic cholinergic processes are required for acquiring sequences.

In a different type of sequential task, Thomas and her colleagues assessed the role of the cholinergic system in a non-verbal working memory task (Thomas et al., 2008). In this task, subjects were administered low doses of scopolamine (0.3 mg s.c.) and were then asked to complete the Groton Maze Learning Test. In this task, subjects were
presented with a 10 x 10 grid on a touch screen in which they were required to find a 28-step hidden pathway. Only their current “move” on the screen was visible, therefore subjects had to rely on their working memory to recall where they had already been in the grid and use trial-and-error techniques to determine the next correct location without overlapping where they had already been. When subjects successfully completed the pathway, they returned to the beginning to find the same pathway again. The results of Thomas et al. (2008) revealed that scopolamine-exposed subjects were severely impaired in their performance on the maze learning task. Further, Thomas et al. (2008) also examined the types of errors made throughout the task and found that scopolamine-exposed subjects made more perseverative errors (making the same error response in succession) and rule-break errors (moving backward, diagonally, or more than one grid box at a time), indicating that working memory was faulty. However, Thomas et al. (2008) explained that these working memory errors cannot be the subjects simply “forgetting the rules”, as they are given consistent feedback when errors are made, but rather an inability to use the rules and adjust their performance accordingly.

Similar results to those observed by Higgins et al (1989), Sitaram et al. (1978), and Thomas et al. (2008) have also been found in studies that have examined the effects of muscarinic cholinergic blockade on the acquisition and retention of learned odor sequences and found that rats given scopolamine showed impaired performance compared to controls (DeRosa & Hasselmo, 2000; Saar et al., 2001). DeRosa and Hasselmo (2000) demonstrated that rats given scopolamine could remember a previously learned odor pair association (A+B-) acquired in the absence of scopolamine,
demonstrating an ability to discriminate between stimuli, scopolamine diminished rats’ abilities to acquire subsequent overlapping (A-C+) and novel odor (D+E-) pairs. Saar and colleagues (2001), however, found that rats given scopolamine in acquisition of odor pairs were impaired in their ability to learn an initial odor pair (A+B-) but were not impaired in learning subsequent novel odor pairs (C+D- and E+F-) relative to controls. Differences in the results between these two investigations may be due to the lack of or presence of scopolamine exposure in the first odor pairing, respectively. Furthermore, in the study conducted by DeRosa and Hasselmo, rats were required to learn a subsequent odor pair that overlapped the initial odor pairing, suggesting that proactive interference may have amplified the effect of scopolamine in that task.

In each of the aforementioned studies of the role of the muscarinic cholinergic system in sequential tasks, deficits have been found in subjects given muscarinic cholinergic antagonists. However, there is an additional sequential task that, when given similar muscarinic cholinergic blockades, does not appear to recruit the neural processes implicated in these other investigations. This task is the serial reaction time (SRT) task (Nissen, Knopman, & Schacter, 1987). In this task, subjects are asked to make responses on a keyboard that correspond with stimuli presented on a computer screen. However, subjects are unaware that the stimuli are presented in a repeating pseudo-random 10-item sequence, thus implicit learning can be assessed as reaction time decreases across four blocks of 100 responses. Subjects then receive a fifth block of 100 randomly presented stimuli which typically causes reaction time to increase relative to performance on the fourth block of the repeated sequence (Knopman & Nissen, 1987; Nissen et al., 1987).
Subjects injected with scopolamine maintained the ability to acquire and perform the procedural SRT task, though overall reaction time was slowed due to peripheral effects of scopolamine (Nissen et al., 1987).

How do we account for the null effects in the SRT task compared to the observations made by the aforementioned sequential tasks? The simplest explanation may be in how performance on these tasks is assessed. The SRT task only looks at reaction time while the other sequential tasks discussed above assessed correct versus incorrect responses. Higgins et al. (1989) assessed error rates and response rates, a measure of speed in the task they claimed to be equivalent to reaction time, and found that overall response rates were decreased under a cholinergic blockade, indicating that subjects were slower to respond under the blockade than under placebo. This overall deficit in speed of performance is similar to that observed by Nissen and colleagues; however, the additional measurement of error rates taken by Higgins et al. provides further explanation for why the speed of performance is slowed – subjects are unable to perform the task correctly. Perhaps if Nissen and colleagues presented error rates in addition to reaction time data, as they have with subsequent studies (Knopman & Nissen, 1991; Nissen & Bullemer, 1987; Nissen, Willingham, & Hartman, 1989), very different conclusions may have been drawn regarding the nature of the effects of scopolamine in the SRT task, as was discovered by Mirza and Stolerman (2000). Mirza and Stolerman (2000) assessed performance in rats trained on a five-choice serial reaction time task (5-CSRTT) given the muscarinic antagonist scopolamine or the nicotinic antagonist mecamylamine. In this task, rats were required to make nose-poke responses at receptacles associated with an illuminated light,
with stimuli presented in a random sequence. Mirza and Stolerman (2000) found that rats were impaired in both drug conditions, but the impairments were differential. For the rats given mecamylamine, the nicotinic antagonist, reaction time was significantly impaired with the highest dose given, 0.5 mg/kg, but overall accuracy was unaffected (Mirza & Stolerman, 2000). In contrast, in rats given scopolamine, the muscarinic antagonist, reaction time was not affected; however, overall accuracy was significantly impaired with the highest dose given, 0.1 mg/kg (Mirza & Stolerman, 2000). Mirza and Stolerman (2000) explain that the differential impairments of scopolamine and mecamylamine reflect two types of issues of information processing with regards to attention. In the 5-CSRTT, rats must make a decision as to what response needs to be made and then choose to make that response. In rats given scopolamine, rats appear to disregard the decision-making process and respond incorrectly, as indicated by overall decreased accuracy (Mirza & Stolerman, 2000). In rats given mecamylamine, rats are making the correct decision, but respond slower in making the correct response (Mirza & Stolerman, 2000). Given this evidence, it is not surprising that Nissen and colleagues did not see an effect of scopolamine in their SRT task because the nature of the impairments caused by scopolamine are related to task accuracy rather than reaction time performance.

A somewhat similar effect was found by Hodges et al. (2009), in which they assessed rats’ performance on the 5-CSRTT as one test among a battery of cognitive tests. In this study, rats that were given scopolamine showed a higher percentage of omission errors, but when rats did make a response their overall accuracy and latency did not differ from control rats. It should be noted, however, that Hodges et al. (2009)
examined several doses of scopolamine, including and exceeding the 0.1 mg/kg dose used by Mirza and Stolerman (2000); and while Hodges et al. (2009) did not report significant differences by dose, the reported figure does show a trend for lower accuracy at the 0.1 mg/kg dose. Nevertheless, according to the results reported by Hodges et al. (2009), it is suggested that in the 5-CSRTT scopolamine may disrupt attention, but moreover the deficits observed are an effect of scopolamine on psychomotor function.

The Effects of Muscarinic Cholinergic Blockade on Rat Serial Pattern Learning and Retention

Previous investigation into the effects of the muscarinic cholinergic antagonist atropine sulfate revealed differential impairments of a sequential pattern, specifically at points in the pattern where structure changed (Chenoweth, 2007). In this series of experiments, two groups of rats, atropine-exposed and saline controls, were trained to acquire a sequential pattern consisting of eight 3-element chunks:

```
123-234-345-456-567-678-781-812
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where the digits represent the clockwise position of levers in a circular array, dashes indicate a 3-s temporal pause, or “phrasing cue” (Fountain, Henne, & Hulse, 1984; Fountain, Rowan, & Carman, 2007; Stempowski, Carman, & Fountain, 1999), and all other intertrial intervals were 1 s. The first element of each chunk is termed a “chunk boundary”, with the following elements in each chunk designated as “within-chunk elements”. The last element of the pattern is called a “violation” element because it violates the rule followed in the previous seven chunks, namely “turn left” after phrasing cues. This pattern was chosen because it is complex yet allows for the use of hierarchical
response rules that can aid in acquisition. Further, the violation element at the end of the pattern allows us to examine rats’ abilities to learn an “exception” to the rule. The goal of the study was to assess if, at what points of the pattern, and how a muscarinic cholinergic antagonist would impair acquisition and performance in this pattern.

Results from the acquisition phase revealed that rats trained on the pattern while receiving daily i.p. injections of 50 mg/kg atropine sulfate showed an overall significant impairment in learning the sequential pattern. These impairments were specific to chunk boundary elements and the violation element, while within-chunk elements were learned at the same rate as those trained on saline. This differential impairment suggests that central cholinergic systems are necessary for encoding the correct responses where structure changes in the pattern, namely chunk boundary elements and the violation element. An extension of the acquisition experiment in which all rats were tested on saline revealed that the effects seen in acquisition persisted even in the absence of atropine, providing further evidence that learning was impaired.

In addition to acquisition of the pattern, retention performance was assessed. The saline control rats in the aforementioned acquisition study were trained to a high criterion of performance, no more than 10% errors on any element type, and were then tested given a dose of 50 mg/kg atropine sulfate. Performance in retention was impaired at the chunk boundary elements and the violation element, with some generalized yet inconsistent disruption in performance at within-chunk elements. This experiment provided evidence that not only are central cholinergic systems necessary for encoding
correct responses where structure changes in a pattern, but also are necessary for accessing already learned responses.

A question that follows this evidence, however, is if these results are unique to the type of pattern learned in this experiment. For example, errors at the first element of the pattern were elevated compared to the other seven chunk boundary elements, indicating that there may be a generalized decrement in performance from errors committed at the violation element. An examination comparing acquisition of a perfect pattern (i.e., 123-234-345-456-567-678-781-812) to that of a violation pattern in the presence of a cholinergic blockade would help to determine how much interference the acquisition of a violation element has on the acquisition of the rest of the pattern.

The final experiment of the series examined the differential effects of two phrasing cue removal probes on performance in atropine-treated versus saline control rats. Prior studies have shown that phrasing cues facilitate learning sequential patterns when they signal structural changes (Fountain et al., 1984; Fountain et al., 2006; Stempowski et al., 1999). In the study by Stempowski et al., rats were trained using a similar sequence production paradigm as Chenoweth (2007), in that rats were required to make responses in a specific sequential pattern for reinforcement within an octagonal chamber. Rats were trained on the same perfect pattern (123-234-345-456-567-678-781-812) with one of three phrasing conditions: no phrasing (all ITI’s were 2.0 s), long-interval phrasing (within-chunk ITI’s were 2.0 s while between chunk ITI’s were 5.0 s), or short-interval phrasing (within-chunk ITI’s were 2.0 s while between chunk
ITI’s were 0.5 s). Rats trained on the long-interval and short-interval conditions acquired the pattern quicker than rats in the no phrasing condition, indicating that having a distinct temporal cue at points in the pattern where structure changes can facilitate acquisition of the pattern. Further, Stempowski et al. (1999) showed that if rats trained with cues are then tested without the cues, performance is severely disrupted, indicating that rats encoded these cues along with the correct lever-press responses of the pattern itself.

Given this evidence, Chenoweth (2007) sought to examine potential differential effects of 2 cue removal probes on atropine exposed vs. saline control performance. The first probe was an Extended Chunk Probe in which the first chunk of the pattern was extended five elements beyond the original three:

12345678

The second probe was a Phrasing Cue Removal probe. This pattern was the same as the Training Pattern with the exception that the 3-s phrasing cues were removed:

1232345456567678781818

In both probe patterns, all intertrial intervals were 1-s; however, the interpattern interval was the same as in the Training Pattern (3 s), so a rat would not know it was in a probe pattern until it reached the serial position of where the first phrasing cue would occur in the training pattern. Therefore, the primary element of interest when looking at the results was what happened at serial position four, the first element that followed a phrasing cue in the Training Pattern.

The results from both probe investigations revealed impaired performance at serial position four in both groups, atropine-treated and saline controls. Of interest are the
types of errors rats committed when presented with the probe patterns. When in a probe pattern, rats trained under saline were more likely to select Lever 2 (49% of errors), the lever that would be considered “correct” in the Training Pattern, or perseverate on Lever 3 (51% of errors). However, whereas rats trained under atropine did make these same errors (17% Lever 2 responses and 37% Lever 3 responses), the remaining 46% of errors were on other random levers within the chamber. A similar pattern was shown with the Phrasing Cue Removal Probe in that rats trained under saline were more likely to perseverate on Lever 3 (33% of errors) or over-extend the chunk by pressing Lever 4 (47% of errors). Likewise, atropine rats made similar errors (35% Lever 3 responses and 26% Lever 4 responses), the remaining 39% of errors were on other random levers within the chamber. Thus, both groups showed evidence of encoding the serial position at which the phrasing cue occurred, as evidenced by the increased number of errors when the cue was removed as compared to the Training Pattern. However, based on the types of errors committed at chunk boundaries when the cues were removed, the results suggest that saline control rats were more organized in their responses whereas atropine rats appear to have had no strategy to find the correct response when normal cues were not present. In other words, saline control rats were able to narrow their responses to within one or two levers surrounding the correct lever (i.e., “Where is the correct lever?”), whereas atropine-treated rats made disorganized responses throughout the entire chamber (i.e., “How do I find the correct lever?”).

The results of the phrasing cue removal probes experiment provided a unique and unpredicted result in terms of the differential impairments shown in the atropine-treated
rats as compared to the saline controls. As such, this type of manipulation may provide an index for investigating numerous issues in sequential learning. First, it may provide evidence for determining if other drugs are working on the same or similar brain systems that are sensitive to central muscarinic cholinergic antagonist. If other drugs show a similar impairment in strategy development, for example, we would have behavioral evidence that similar brain systems were impaired. Differential impairments, likewise, would provide evidence that multiple brain systems are involved in acquiring and retaining sequential pattern structure.

Another route of investigation using this type of manipulation may be useful in determining if the removal or addition of a pharmacological blockade differentially affects rats’ abilities to develop efficient strategies to make correct responses in the absence of temporal cues. Will prior learning to a high criterion without a cholinergic blockade, for example, produce a savings effect in rats given a cholinergic blockade combined with temporal cue removal? Likewise, will impaired learning due to a cholinergic blockade prevent the development and utilization of efficient strategies in the absence of temporal cues when the drug is no longer present? Simply put, would prior learning in a specific state (i.e., drug vs. no drug) affect performance in a reversal learning task? The present study sought to answer these questions.

**Scopolamine Effects with Water Reward in Rat Serial Pattern Learning**

The prior research involving a muscarinic cholinergic antagonist produced evidence for the role of central cholinergic systems in the acquisition of a serial pattern containing a violation element. This previous research utilized a procedure involving
brain stimulation reward (BSR) to reinforce rats when making correct responses. Using a BSR procedure is ideal for studies in smaller study designs, as it allows for a substantial amount of data to be collected within a short amount of time, but can become difficult for larger designs requiring a larger number of subjects and also requires specific surgical and testing equipment and training that may not be readily available for some researchers. Therefore, an appetitive water reinforcement procedure is often a more appealing and practical alternative to a BSR procedure.

It should also be noted that while atropine and scopolamine both block muscarinic cholinergic receptors, scopolamine is significantly more potent and thus requires a lower dose to effectively cross the blood-brain barrier and saturate muscarinic receptors compared to atropine (Sipos, Burchnell, & Galbicka, 1999). However, there is conflicting evidence in the literature suggesting that rats may or may not perform for water reinforcement when given scopolamine (Fisher, 1962; Kuribara, 1988; Miczek & Lau, 1975; Morley & Russin, 1978; Russell, Ehlert, & Hwa, 1986; Sanger, 1976). The literature suggests that rats with acute exposure to scopolamine have difficulty performing tasks that use water as a reinforcement; however, in rats given chronic exposure, rats can overcome this impediment and perform water-reinforcement tasks with little or no difficulty. Given this evidence, as well as the consideration of the complexity of the octagonal chamber paradigm, a pilot study was conducted to investigate the possibility of using water reinforcement in rats given scopolamine.

Eight Long-Evans hooded rats were shaped to make nose-poke responses for water reinforcement, first without scopolamine and then under daily injections of
scopolamine (0.6 mg/kg) given 30 min prior to each shaping session. On average, rats took approximately 1 – 3 daily shaping sessions to reach the criterion of making 240 nose-poke responses under scopolamine. Once rats reached criteria, they were transferred to an octagonal testing chamber to learn a serial pattern containing a violation element: 123-234-345-456-567-678-781-818, where digits represent the nose-poke receptacles numbered clockwise, dashes represent 3-s phrasing cues, and all other ITI’s were 1 s. Rats received 10 patterns a day for 6 days, while also receiving daily injections of scopolamine 30 min prior to each test session.

Results of this pilot study indicated that rats are capable of working for water reinforcement in the octagonal chamber paradigm under chronic exposure to scopolamine. Data analysis also revealed that rats given scopolamine showed deficits at the same points in the pattern as atropine rats in the previous study. Granted, these results were only after 6 days of acquisition, but there was a clear difference in performance between within-chunk versus chunk boundaries and the violation element, which is in-line with the deficits observed in the atropine investigation. Proportions of types of errors differed slightly from what was observed in the atropine study, though in an extended investigation, the proportions of types of errors may differ from prior observations. In sum, the results of the pilot study were encouraging and provided a viable alternative in methodology for the present study that allowed for a decrease in the overall number of animals required to complete the present study without compromising the ability to compare the results of the present study to prior research into the role of central cholinergic systems in sequential learning.
Specific Aims

The present study describes two experiments that explored the effects of scopolamine, a muscarinic cholinergic antagonist, on rat serial pattern learning. The first experiment compared acquisition of a perfect versus violation pattern in rats given either scopolamine or saline. This experiment provided evidence as to how cholinergic systems are involved in rats’ abilities to learn serial patterns with or without a violation element. As noted above, the violation element was hypothesized to be a source of interference in the acquisition of other pattern elements, whereas in a perfect pattern this source of potential interference or contamination is absent. By comparing acquisition of these two types of patterns in the presence of scopolamine or saline, we were able to assess if the violation element interferes with acquisition of the other elements of the pattern.

The second experiment utilized a reversal learning task that is a variation of the above described phrasing cue removal manipulation that was tested in the atropine study. In this task, rats from Experiment 1 were transferred to an unphrased version of the pattern experienced in Experiment 1. Further, rats were transferred to either a same or different drug condition than what was experienced in Experiment 1 to allow for an examination of the effects of prior versus current drug state on rats’ abilities to learn the reversal learning task.
Experiment 1

The purpose of Experiment 1 was to examine the effects of scopolamine, a muscarinic cholinergic antagonist, on the acquisition of a “perfect” versus “violation pattern:


Both patterns contain transitions between chunks (i.e., chunk boundary elements) using a sequential production paradigm; however, the violation pattern contains an element at the end of the pattern that violates the rules set forth in the rest of the pattern. It was hypothesized that this violation element may be a source of interference in the acquisition of the pattern, making it more difficult for rats to acquire the remaining elements of the pattern relative to rats learning a perfect pattern. By parsing out the effects of pattern acquisition based on the presence or absence of this violation element, we were able to examine the extent to which the muscarinic cholinergic system is involved in the acquisition of the different pattern element types.

Method

Subjects. Forty-eight naïve Long Evans hood rats (Rattus norvegicus) at least 90 days old served as subjects. Rats were housed individually with food freely available in the home cage and were water deprived throughout behavioral testing. Rats showing any signs of dehydration (loss of skin elasticity, weight loss greater than 85% of their free-
feeding weight, listlessness, or unkempt appearance) were provided supplementary water as needed. All rats were kept on a 15:9-h light-dark cycle, with testing occurring during the light portion of the cycle.

**Apparatus.** Four chambers approximately 15 x 30 x 30 cm (Fountain & Rowan, 1995a; 1995b) equipped with a nose poke receptacle 5 cm above the floor on one wall with a solenoid attached to deliver a water reinforcement of 0.025 ml were used for shaping. The chambers were constructed of clear Plexiglas with hardware cloth for the floor, and individually enclosed in sound-attenuating cabinets made of particle board (20 x 60 x 65 cm). Nose poke receptacles were 2.5-cm diameter PVC pipe end caps painted flat black with infrared emitter-detector pairs mounted on the sides and an LED cue light in the rear.

Six octagonal chambers (40 cm between parallel walls x 30 cm tall; Fountain et al., 1995a; 1995b) constructed of clear Plexiglas with a nose poke receptacle (same as above described) mounted 5 cm above the floor on each wall and a floor of stainless steel hardware cloth served as testing chambers in the experiment (see Figure 1, top panel). Chambers were located in individual sound-attenuating cabinets within the same room and controlled from an adjoining room with a microcomputer and interface (Med Associates, Inc., Fairfield, VT). All shaping and test chambers were monitored using closed-circuit cameras mounted above the chambers.

**Drugs.** Rats in the scopolamine-treated groups were given daily i.p. injections of 0.6 mg/kg scopolamine hydrobromide (Sigma) dissolved in saline. Rats in the control
Figure 1:  

*Top Panel:* An octagonal operant chamber equipped with nose poke receptacles on each wall. *Bottom Panel:* Schematic representation (top view) of an octagonal chamber with receptacles numbered clockwise 1-8.
Figure 1
groups were given i.p. injections of an equivalent volume of saline (i.e., 1 ml/kg). All injections were given 30 min prior to daily training sessions.

**Procedure.** Rats began the shaping procedure after approximately 2 days of water deprivation. Rats were shaped to nose poke for water reinforcement in the above described shaping chamber. In the first nose poke shaping session, the receptacle light was illuminated throughout the entire session and extinguished when the rat made 240 nose poke responses. Once the rat met this criterion, the following day it was shaped to make discrete nose-poke responses. In the discrete shaping procedure, the light in the receptacle was illuminated at the beginning of each trial and, when the rat responded, extinguished and water reinforcement was delivered. A 1-s intertrial interval separated trials. Rats were given up to 2 sessions to meet a criterion of 240 responses in order to remain in the experiment. Rats that met the shaping criterion were then randomly assigned to one of two drug conditions: Scopolamine or Saline. All rats were then given up to 3 additional discrete shaping sessions under their respective drug conditions to make 240 responses. Rats that failed to meet criterion at any stage within the shaping procedure were replaced with new rats for a total of 48 rats to be maintained.

Once rats achieved the shaping criterion under their respective drug conditions, they were randomly assigned to one of two pattern types per drug condition: Perfect-Saline (N = 12), Perfect-Scopolamine (N = 12), Violation-Saline (N = 12), or Violation-Scopolamine (N = 12). Rats then began daily training sessions on their patterns:


where digits represent the clockwise position of the receptacles in the chamber (see Figure 1, bottom panel). Dashes represent 3-s temporal pauses, or “phrasing cues”, and all other intertrial intervals were 1 s. The patterns were presented as a discrete-trial, eight-choice procedure with correction in which all eight receptacles were illuminated at the beginning of each trial. If the rat made a correct choice, all receptacle lights extinguished and the rat received water reinforcement. If the rat made an incorrect choice, all receptacle lights except the correct receptacle light extinguished and water was given once the rat made the correct nose poke response. Nose poke responses and latency to the first response were recorded for each trial to assess performance. Rats received 10 patterns each day for 49 days.

Results

In all reported analyses, main effects and interactions were considered significant if \( p < 0.05 \).

**Acquisition of element types.** Analyses to determine differential effects of cholinergic blockade, as well as potential differences between pattern types, on the various elements of the pattern were conducted. Figure 2 shows the mean percentage of chunk boundary element errors for all four groups over the 49 days of acquisition. Results show that scopolamine impaired acquisition of the chunk boundary elements, but pattern type did not interact with drug exposure. A 2(Pattern) X 2(Drug) X 49(Day) mixed design analysis of variance (ANOVA) was conducted on rats’ daily mean percentage of chunk boundary element errors. Main effects were found for drug, \( F(1, 44) \)
Figure 2: Acquisition curves for chunk boundary elements for Perfect-Saline, Perfect-Scopolamine, Violation-Saline, and Violation-Scopolamine groups over the 49 days of acquisition in Experiment 1. Daily mean percentage of errors was averaged across all chunk boundary elements of the patterns. Error bars: ± SEM.
Figure 2.

Mean % Chunk Boundary Element Errors

- Perfect-Saline
- Perfect-Scopolamine
- Violation-Saline
- Violation-Scopolamine
= 51.49, \eta^2_p = 0.54, and day, \text{F}(48, 2112) = 118.74, \eta^2_p = 0.73, and the Drug X Day interaction was significant, \text{F}(48, 2112) = 3.76, \eta^2_p = 0.08. Planned comparisons showed that scopolamine-exposed rats produced significantly more chunk boundary element errors than saline controls on Days 3-49.

Figure 3 shows the mean percentage of within-chunk element errors for all four groups over the 49 days of acquisition. Results show that scopolamine interfered with rats’ abilities to learn the within-chunk elements of the pattern, and pattern type interacted to produce a transient deficit in Violation-Scopolamine group performance. A 2(Pattern) X 2(Drug) X 49(Day) mixed design ANOVA was conducted on rats’ daily mean percentage of within-chunk element errors. Main effects were found for drug, \text{F}(1, 44) = 78.46, \eta^2_p = 0.64, and day, \text{F}(48, 2112) = 32.17, \eta^2_p = 0.42, the Drug X Day interaction was significant, \text{F}(48, 2112) = 4.38, \eta^2_p = 0.09, and the Pattern X Day interaction approached significance, \text{F}(48, 2112) = 1.35, p = 0.058, \eta^2_p = 0.03. Planned comparisons showed that scopolamine-exposed rats produced significantly more within-chunk element errors than saline controls on Days 1-2 and 5-49. There were no consistent differences between rats trained on the Perfect pattern and rats trained on the Violation pattern.

Lastly, Figure 4 shows the mean percentage of violation element errors for the Violation-Saline and Violation-Scopolamine groups over the 49 days of acquisition. Results show that scopolamine impaired acquisition of the violation element. A 2(Drug) X 49(Day) repeated measures ANOVA was conducted on rats’ daily mean percentage of
Figure 3: Acquisition curves for within-chunk elements for Perfect-Saline, Perfect-Scopolamine, Violation-Saline, and Violation-Scopolamine groups over the 49 days of acquisition in Experiment 1. Daily mean percentage of errors was averaged across all within-chunk elements of the patterns. Error bars: ± SEM.
Figure 3.

Day 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49

Mean % Within-chunk Element Errors

Perfect-Saline
Perfect-Scopolamine
Violation-Saline
Violation-Scopolamine
Figure 4: Acquisition curves for the violation element for the Violation-Saline and Violation-Scopolamine groups over the 49 days of acquisition in Experiment 1. Error bars: ± SEM
Figure 4.
violation element errors. Main effects were found for drug, $F(1, 22) = 11.78, \eta_p^2 = 0.35$, and day, $F(48, 1056) = 12.27, \eta_p^2 = 0.36$, and the Drug X Day interaction was significant, $F(48, 1056) = 18.65, \eta_p^2 = 0.46$. Planned comparisons showed that while saline-exposed rats initially showed significantly impaired performance on Days 6, 8-10, and 12-13, saline controls were able to acquire the correct violation element response while scopolamine-exposed rats continued to produce significantly more errors than saline controls on Days 23-49.

**Last day of acquisition and error analyses.** Figure 5 shows the mean percentage of errors for all four groups pooled across all 10 patterns on the last day of acquisition (Day 49). Results show that scopolamine interfered with acquisition of chunk boundary elements, the violation element, and in select within-chunk elements. A 2(Pattern) X 2(Drug) X 8(Chunk) X 3(Element) mixed design ANOVA was conducted on rats’ mean percentage of errors (pooled across all 10 patterns on Day 49 of acquisition). The ANOVA indicated significant main effects for pattern, $F(1, 44) = 6.35, \eta_p^2 = 0.13$ and drug, $F(1, 44) = 55.65, \eta_p^2 = 0.56$, and the Pattern X Drug interaction approached significance, $F(1, 44) = 3.58, p = 0.065, \eta_p^2 = 0.08$.

Mauchly’s test indicated that the assumption of sphericity had been violated for Chunk (chi-square = 44.01), therefore the degrees of freedom were corrected using Huynh-Feldt estimates of sphericity (epsilon = 0.93). The ANOVA indicated a significant main effect for chunk, $F(6.50, 285.86) = 5.18, \eta_p^2 = 0.11$, and significant interactions for Pattern X Chunk, $F(6.50, 285.86) = 4.45, \eta_p^2 = 0.09$, Drug X Chunk, $F(6.50, 285.86) = 3.65, \eta_p^2 = 0.08$, and Pattern X Drug X Chunk, $F(6.50, 285.86) = 4.76, \eta_p^2 = 0.10$. 
Figure 5: Mean percentage of trial x trial errors for Perfect-Saline, Perfect-Scopolamine, Violation-Saline, and Violation-Scopolamine groups on the last day of acquisition (Day 49) in Experiment 1. Percentages of errors were pooled across the 10 patterns of the last day of acquisition.
Figure 5.

The graph illustrates mean percentage errors across different patterns for four conditions: Perfect-Saline, Perfect-Scopolamine, Violation-Saline, and Violation-Scopolamine. The x-axis represents the pattern numbers from 1 to 8, and the y-axis shows the mean percentage errors ranging from 0% to 100%. The legend indicates the symbols for each condition.
Mauchly’s test also indicated that the assumption of sphericity had been violated for Element (chi-square = 31.77), therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (epsilon = 0.66). The ANOVA indicated a significant main effect for element, $F(1.31, 57.81) = 18.66$, $\eta_p^2 = 0.30$, and significant interactions for Pattern X Element, $F(1.31, 57.81) = 6.34$, $\eta_p^2 = 0.13$, and Drug X Element, $F(1.31, 57.81) = 7.40$, $\eta_p^2 = 0.14$.

Mauchly’s test also indicated that the assumption of sphericity had been violated for Chunk X Element (chi-square = 247.03), therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (epsilon = 0.51). The ANOVA indicated significant interactions for Chunk X Element, $F(7.09, 311.73) = 5.97$, $\eta_p^2 = 0.12$, Pattern X Chunk X Element, $F(7.09, 311.73) = 6.76$, $\eta_p^2 = 0.13$, Drug X Chunk X Element, $F(7.09, 311.73) = 3.34$, $\eta_p^2 = 0.07$, and Pattern X Drug X Chunk X Element, $F(7.09, 311.73) = 3.88$, $\eta_p^2 = 0.08$.

Planned comparisons showed that scopolamine-exposed rats produced more errors than saline controls on all chunk boundary elements, as well as the final element of the pattern (in the Violation groups, this element of the pattern was the violation element). Additionally, Violation-Scopolamine rats produced significantly more errors than Violation-Saline rats on the 3rd element of chunks 3-6. Further, Violation-Scopolamine rats produced significantly more errors than Perfect-Scopolamine rats on the 3rd element of chunks 1, 3, 7, and 8. There were no differences across chunks between the Violation-Saline and Perfect-Saline groups.
The types of errors committed on the last day of acquisition were also analyzed. Figure 6 shows the proportion of error type committed at chunk boundary, within-chunk, and violation elements for each group. As shown in the top panel of Figure 6, all four groups showed a consistently higher proportion of perseveration errors (i.e., choosing the previously reinforced receptacle, as in 123-3) than any other error type at chunk boundary elements. The “back 2 receptacles” error (i.e., choosing the receptacle a position one to the left of the correct receptacle, as in 123-1) appears to be drug-dependent, with scopolamine-exposed rats committing a higher proportion of this error than rats that received saline. There are no clear differences between drug or pattern conditions for the remaining two error types, overextensions (i.e., choosing the receptacle that extends the previous chunk, as in 123-4) and other errors (i.e., choosing an incorrect receptacle that does not fall into one of the other three error categories, as in 123-6).

Differences in error types were also present in the within-chunk elements. As shown in the middle panel of Figure 6, the majority of errors across groups were either perseveration (e.g., 122) or back 2 receptacles (e.g., 121) errors, with rats in the Violation-Scopolamine group performing a higher proportion of perseveration errors than the other three groups. The within-chunk element error types were further analyzed by position within the chunk – 2\textsuperscript{nd} element and 3\textsuperscript{rd} element of chunks – for the Perfect-Scopolamine and Violation-Scopolamine groups. For the 2\textsuperscript{nd} element of chunks, both the Perfect-Scopolamine and the Violation-Scopolamine groups were more likely to commit perseveration errors (57.3\% and 79.2\%, respectively), while on the 3\textsuperscript{rd} element of chunks the Perfect-Scopolamine group switched to back 2 receptacles errors (49.9\%) and the
Figure 6: 

*Top panel:* Mean percentage of error types at chunk boundary elements for Perfect-Saline, Perfect-Scopolamine, Violation-Saline, and Violation-Scopolamine groups. Proportions of errors were averaged across all chunk boundary elements for the 10 patterns on the last day of acquisition of Experiment 1. 

*Middle panel:* Mean percentage of error types at within-chunk elements for Perfect-Saline, Perfect-Scopolamine, Violation-Saline, and Violation-Scopolamine groups. Proportions of errors were averaged across all within-chunk elements for the 10 patterns on the last day of acquisition of Experiment 1. 

*Bottom panel:* Mean percentage of error types at the violation element for Violation-Saline and Violation-Scopolamine groups. Proportions of errors were averaged across all violation elements for the 10 patterns on the last day of acquisition of Experiment 1.
Figure 6.
Violation-Scopolamine group was equally likely to commit a perseveration or back 2 receptacles error (50% and 46.2%, respectively).

Lastly, the error types committed on the violation element were analyzed. There were no differences between the Violation-Saline and Violation-Scopolamine groups for the types of errors committed, with both groups showing a high proportion of overextension errors (e.g., 812) than any other error type (see Figure 6, bottom panel).

Discussion

The results of Experiment 1 clearly demonstrate that the cholinergic system is necessary to acquire sequential patterns, as evidenced by the impaired acquisition of the Perfect-Scopolamine and Violation-Scopolamine groups compared to the Perfect-Saline and Violation-Saline groups. Further, while rats showed transient impairments by pattern type, the evidence is fairly weak that the presence of a violation element interacted significantly with the presence of scopolamine to produce a reliable impairment of pattern acquisition. However, there is some evidence to suggest a difference in how the rats are learning their respective patterns by examining the types of errors made by the Perfect-Scopolamine and Violation-Scopolamine groups. As shown in Figure 6, these two groups appear to be using fairly similar strategies to acquire the chunk boundary elements, evidenced by the similarities in the types of errors the two groups are making (see Figure 6, top panel); however, there does appear to be a difference in the strategies used to learn the within-chunk elements, made clear by the higher proportion of perseveration errors committed by the Violation-Scopolamine group (see Figure 6,
middle panel). Alternatively, rats in the Perfect-Scopolamine group were more likely to use similar strategies as the two saline groups in acquiring the within-chunk elements, i.e., they were equally likely to commit a back 2 receptacles or a perseveration response (see Figure 6, middle panel).

By examining the error types by position within the chunk, we are able to see a clear difference in strategy shifting in the Perfect-Scopolamine and Violation-Scopolamine groups. Recall that on the 2nd element of chunks rats in both groups were more likely to commit a perseveration error, and on the 3rd element of chunks rats in both groups adopted a comparatively higher proportion of back 2 receptacles errors. One might argue that rats in the Violation-Scopolamine group are making the back 2 receptacles response on the 3rd element of chunks in anticipation of the violation element at the end of the pattern; however, this argument cannot account for this same error observed at a similar proportion in the Perfect-Scopolamine group. An explanation that can account for this behavior in both groups is that when rats reach the 3rd element of the chunk the strategy shifts to a preparatory chunk boundary element response (i.e., “turn left”).

This explanation leads to an additional question: If rats in both scopolamine groups are making preparatory “chunk-boundary-like” responses before the phrasing cue, are they encoding the phrasing cues in the same way as rats that received saline in acquisition? Moreover, will we observe differential impairments if the phrasing cues are removed? Experiment 2 sought to answer this question by transferring rats to unphrased patterns, as well as to a same or different drug condition.
Experiment 2

Prior research has indicated that phrasing cues at chunk boundaries facilitate learning sequential patterns (Fountain et al., 1984; Stempowski et al., 1999). Further, if rats trained with phrasing cues are later presented the same pattern without the cues, performance is severely disrupted, particularly at chunk boundary elements (Stempowski et al., 1999). Additional unpublished research utilizing the same paradigm as the present study in which phrasing cues were removed has suggested that rats may be encoding serial position as an occasion setter for the use of temporal phrasing cues at chunk boundaries. In this study, rats were trained on serial patterns similar to the patterns in the present study composed of 3 or 4 element chunks separated by temporal phrasing cues. Rats then experience probe trials in which they experienced a pattern where the phrasing cues of their original training pattern were removed and the extended chunk probe used in the Chenoweth (2007) study: 12345678. This study, like that of Stempowski and colleagues (1999), found that by removing the phrasing cues, rats are unable to accurately predict the correct response, not unlike the behavior of rats in which an occasion setter is absent in a more traditional Pavlovian conditioning paradigm.

In Experiment 2, rats were transferred to a pattern that contained the same pattern of responses as received in training; however, all phrasing cues were removed, leaving a 1-s intertrial interval throughout the pattern. Based on the prior research mentioned
above, it would be reasonable to expect a disruption in performance at the chunk boundary elements following the missing phrasing cues in the saline control groups. Additionally, evidence from prior research with a muscarinic cholinergic antagonist shows a differential impaired performance at chunk boundary elements as well, in that rats given atropine showed a severely impaired ability to develop efficient strategies to find the correct responses at chunk boundary elements (Chenoweth, 2007). In this respect, Experiment 2 provided an opportunity to partially replicate the results observed by Chenoweth (2007), adding strength to the argument that central cholinergic systems are involved in strategy development when cues are removed, not only in a violation pattern, but also in a perfect pattern. Furthermore, the hippocampus, a structure that receives cholinergic input from the medial septum has been implicated in occasion setting (Clarke, Skinner, & van der, 2001; Everitt & Robbins, 1997; Holland, Lamoureux, Han, & Gallagher, 1999; Schmajuk & Buhusi, 1997). Therefore, it might be expected that by depleting this cholinergic input, rats would have an increased difficulty in performing in the absence of cues that once predicted a correct response to be made at that serial position.

Considering this prior evidence suggesting the potential role of the cholinergic system in the ability to perform in the absence of cues, rats were transferred to either the same or different drug condition as received in acquisition training in addition to having phrasing cues removed from their training patterns. This transfer allowed for an evaluation of whether prior experience under a different drug condition affects reversal learning. In an investigation by Ragozzino and Choi (2004), levels of acetylcholine
output from the medial striatum, a structure implicated in learning changes to task contingencies, were measured in a reversal learning task. In this task, researchers trained rats on a place discrimination task in which rats were required to learn to enter a correct arm from a four-arm cross maze for reinforcement; rats were then required to enter the opposite arm in reversal learning. The researchers found an increase in acetylcholine levels in the reversal learning task compared to initial acquisition. These results suggest that cholinergic systems may play a key role in the acquisition of a reversal learning task. By introducing a cholinergic blockade during reversal learning to rats who have mastered the original task under saline, we were able to examine the extent to which central cholinergic systems are necessary in the present sequential learning task.

Method

Subjects. Subjects were the same as in Experiment 1. One rat was lost on Day 2.

Apparatus. The apparatus was the same as in Experiment 1.

Drugs. Drug preparation and injections were the same as in Experiment 1.

Procedure. The procedure was the same as in Experiment 1 with the exception that rats were transferred to a new group to form eight groups total (Pattern Type – Training Drug Condition – Transfer Drug Condition): Perfect-Saline-Saline (N = 6), Perfect-Saline-Scopolamine (N = 6 for Day 1; N = 5 for Days 2-14), Perfect-Scopolamine-Saline (N = 6), Perfect-Scopolamine-Scopolamine (N = 6), Violation-Saline-Saline (N = 6), Violation-Saline-Scopolamine (N = 6), Violation-Scopolamine-Saline (N = 6), and Violation-Scopolamine-Scopolamine (N = 6). Further, the Perfect and
Violation patterns contained no phrasing cues, instead having a 1-s intertrial interval throughout the entire pattern:

Perfect: 12323435456567678781812

Violation: 12323435456567678781818

Nose poke responses and latency to the first response were recorded for each trial to assess performance. Rats received 10 patterns each day for 14 days, allowing for rats to attain asymptotic levels of performance.

**Results**

In all reported analyses, main effects and interactions were considered significant if $p < 0.05$.

**Analysis of element types for first day of transfer.** Figure 7 shows the mean percentage of chunk boundary element errors for the last day of acquisition and the first day of transfer. The results show differential disruptions in performance among the groups. A 2(Pattern) X 2(Acquisition Drug) X 2(Transfer Drug) X 2(Day) mixed design ANOVA was conducted on rats’ mean percentage of chunk boundary element errors. Main effects were found for acquisition drug, $F(1, 40) = 30.87, \eta_p^2 = 0.44$, and day, $F(1, 40) = 74.49, \eta_p^2 = 0.65$, and significant interactions were found for Acquisition Drug X Transfer Drug, $F(1, 40) = 16.64, \eta_p^2 = 0.29$, Transfer Drug X Day, $F(1, 40) = 11.24, \eta_p^2 = 0.22$, and Acquisition Drug X Transfer Drug X Day, $F(1, 40) = 13.16, \eta_p^2 = 0.25$.

Planned comparisons showed an effect of phrasing cue removal transfer that was dependent upon drug transfer condition for rats that received saline in acquisition (see Figure 7, top panel). Rats that were given saline in acquisition showed a significant
Figure 7:  

*Top panel:* Mean percentage of chunk boundary element errors for Perfect-Saline-Saline, Perfect-Saline-Scopolamine, Violation-Saline-Saline, and Violation-Saline-Scopolamine groups for the last day of acquisition (Experiment 1, Day 49) and the first day of transfer (Experiment 2, Day 1). Daily mean percentage of errors was averaged across all chunk boundary elements of the patterns. *Bottom panel:* Mean percentage of chunk boundary elements errors for Perfect-Scopolamine-Scopolamine, Perfect-Scopolamine-Saline, Violation-Scopolamine-Scopolamine, and Violation-Scopolamine-Saline groups for the last day of acquisition (Experiment 1, Day 49) and the first day of transfer (Experiment 2, Day 1). Daily mean percentage of errors was averaged across all chunk boundary elements of the patterns. Error bars: ± SEM. Closed symbols indicate rats stayed on same drug as in acquisition; open symbols indicate rats transferred to different drug.
Figure 7.

Acquisition Transfer

Mean % Chunk Boundary Element Errors

0 10 20 30 40 50 60 70 80 90 100

Perfect-Saline-Saline
Perfect-Saline-Scopolamine
Violation-Saline-Saline
Violation-Saline-Scopolamine

Phase
increase in chunk boundary element errors if they were transferred to scopolamine, regardless of pattern type. Additionally, rats that received scopolamine in acquisition showed a significant increase in chunk boundary element errors in transfer regardless of pattern or drug transfer condition (see Figure 7, bottom panel).

Figure 8 shows the mean percentage of within-chunk element errors for the last day of acquisition and the first day of transfer. The results show differential disruptions in performance among the groups. A 2(Pattern) X 2(Acquisition Drug) X 2(Transfer Drug) X 2(Day) mixed design ANOVA was conducted on rats’ mean percentage of within-chunk element errors. Main effects were found for acquisition drug, $F(1, 40) = 15.08$, $\eta_p^2 = 0.27$, and day, $F(1, 40) = 23.09$, $\eta_p^2 = 0.37$, and significant interactions were found for Acquisition Drug X Day, $F(1, 40) = 13.92$, $\eta_p^2 = 0.26$, and Acquisition Drug X Transfer Drug X Day, $F(1, 40) = 16.89$, $\eta_p^2 = 0.30$. Planned comparisons showed an effect of drug for rats that received saline in acquisition and transferring to scopolamine showing a significant impairment of performance on within-chunk elements from acquisition to transfer regardless of pattern type (see Figure 8, top panel). Additionally, rats trained on scopolamine in acquisition and transferred to saline showed a significant increase in within-chunk element errors, but rats that were trained on scopolamine and remained on scopolamine did not increase their within-chunk element errors when phrasing cues were removed (see Figure 8, bottom panel).

Lastly, Figure 9 shows the mean percentage of violation element errors for the last day of acquisition and the first day of transfer. The results show a differential effect of the phrasing cue removal among the groups. A 2(Acquisition Drug) X 2(Transfer Drug)
Figure 8:  

*Top panel:* Mean percentage of within-chunk element errors for Perfect-Saline-Saline, Perfect-Saline-Scopolamine, Violation-Saline-Saline, and Violation-Saline-Scopolamine groups for the last day of acquisition (Experiment 1, Day 49) and the first day of transfer (Experiment 2, Day 1). Daily mean percentage of errors was averaged across all within-chunk elements of the patterns.  

*Bottom panel:* Mean percentage of within-chunk elements errors for Perfect-Scopolamine-Scopolamine, Perfect-Scopolamine-Saline, Violation-Scopolamine-Scopolamine, and Violation-Scopolamine-Saline groups for the last day of acquisition (Experiment 1, Day 49) and the first day of transfer (Experiment 2, Day 1). Daily mean percentage of errors was averaged across all within-chunk elements of the patterns.  

Error bars: ± SEM. Closed symbols indicate rats stayed on same drug as in acquisition; open symbols indicate rats transferred to different drug.
Figure 8.

![Graph showing mean % within-chunk element errors across acquisition and transfer phases for different conditions.](image-url)
Figure 9: Top Panel: Mean percentage of violation element errors for Violation-Saline, Violation-Saline-Scopolamine, Violation-Scopolamine-Scopolamine, and Violation-Scopolamine-Saline groups for the last day of acquisition (Experiment 1, Day 49) and the first day of transfer (Experiment 2, Day 1). Error bars: ± SEM. Closed symbols indicate rats stayed on same drug as in acquisition; open symbols indicate rats transferred to different drug.

Bottom Panel: Re-plotted data from the Top Panel above. Mean percentage of violation element errors for rats receiving saline in acquisition (closed circles) and rats receiving scopolamine in acquisition (open triangles) for the last day of acquisition (Experiment 1, Day 49) and the first day of transfer (Experiment 2, Day 1). Error bars: ± SEM.
Figure 9.

The figure illustrates the mean % violation element errors across different combinations of acquisition and transfer phases.

- **Violation-Saline-Saline**
- **Violation-Saline-Scop**
- **Violation-Scop-Scop**
- **Violation-Scop-Saline**

The top graph shows the data for different phases of acquisition and transfer, with error bars indicating variability.

The bottom graph focuses on the comparison between saline and scopolamine conditions during the acquisition phase.
X 2(Day) repeated measures ANOVA was conducted on rats’ mean percentage of violation element errors. Main effects were found for acquisition drug, $F(1, 20) = 52.12$, $\eta_p^2 = 0.72$, and day, $F(1, 20) = 14.73$, $\eta_p^2 = 0.42$, and the Acquisition Drug X Day interaction was significant, $F(1, 20) = 17.99$, $\eta_p^2 = 0.47$. A post-hoc t-test analysis based on the error term from the ANOVA indicated an effect of the transfer with rats that received saline in acquisition showing a significant increase in violation element errors regardless of whether they remained on saline or transferred to scopolamine. Rats that received scopolamine in acquisition showed no difference in violation element errors in transfer. Furthermore, rats that received saline in acquisition do show a significantly lower rate of errors in transfer than rats that received scopolamine in acquisition, indicating that rats that received saline in acquisition were not at ceiling in transfer.

**Error analyses of first day of transfer.** The types of errors committed on the first day of transfer were analyzed and compared to those committed on the last day of acquisition (Experiment 1, Day 49). Figure 10 shows the proportion of error type committed at chunk boundary elements. All groups showed a consistently high proportion of overextension errors at chunk boundary elements. This is in contrast to the last day of acquisition in which all groups showed bias towards perseveration errors (see Figure 6, top panel). Only the Violation-Saline-Saline group appeared to maintain the perseveration error bias at chunk boundary elements (see Figure 10, top panel). Another change of note is the lack of “other” errors at chunk boundary elements in both the Perfect-Saline-Saline and Violation-Saline-Saline groups on first day of transfer, as compared to the last day of acquisition.
Figure 10: Top panel: Mean percentage of error types at chunk boundary elements for Perfect-Saline-Saline, Perfect-Saline-Scopolamine, Violation-Saline-Saline, and Violation-Saline-Scopolamine groups. Percentages of errors were averaged across all chunk boundary elements for the 10 patterns on the first day of transfer of Experiment 2. Bottom panel: Mean percentage of error types at chunk boundary elements for Perfect-Scopolamine-Scopolamine, Perfect-Scopolamine-Saline, Violation-Scopolamine-Scopolamine, and Violation-Scopolamine-Saline groups. Percentages of errors were averaged across all chunk boundary elements for the 10 patterns on the first day of transfer of Experiment 2.
Figure 10.
Differences in error types were also present in the within-chunk elements. As shown in Figure 11, while most groups maintained the relatively high proportion of perseveration errors they committed on the last day of acquisition, there were differential shifts in the proportion of the back 2 receptacles errors committed at within-chunk elements on the first day of transfer. For example, the Perfect-Saline-Saline group committed significantly fewer back 2 receptacles errors in transfer than on the last day of acquisition (see Figure 11, top panel), while the Violation-Scopolamine-Saline group committed significantly more back 2 receptacles errors in transfer than on the last day of acquisition (see Figure 11, bottom panel). As in the analysis of within-chunk element of errors for the last day of acquisition, the within-chunk element error types were further analyzed by position within the chunk – 2nd element and 3rd element of chunks. However, unlike what was observed on the last day of acquisition, there was not a reliable difference in the types of errors committed at the 2nd element versus the 3rd element of chunks; rats tended to maintain a similar proportion of error type across the two element positions.

Lastly, the error types committed on the violation element were analyzed. In general, there were no significant changes in the types of errors committed at the violation element on the first day of transfer compared to the last day of acquisition; rats committed a high proportion of overextension in errors on both days. However, a notable change was the increase of noise with a small proportion of other errors committed by the Violation-Saline-Scopolamine, Violation-Scopolamine-Scopolamine, and Violation-Scopolamine-Saline groups, as well as a small proportion of back 2 receptacles errors.
Figure 11:  *Top panel:* Mean percentage of error types at within-chunk elements for Perfect-Saline-Saline, Perfect-Saline-Scopolamine, Violation-Saline-Saline, and Violation-Saline-Scopolamine groups. Percentages of errors were averaged across all within-chunk elements for the 10 patterns on the first day of transfer of Experiment 2.  *Bottom panel:* Mean percentage of error types at within-chunk elements for Perfect-Scopolamine-Scopolamine, Perfect-Scopolamine-Saline, Violation-Scopolamine-Scopolamine, and Violation-Scopolamine-Saline groups. Percentages of errors were averaged across all within-chunk elements for the 10 patterns on the first day of transfer of Experiment 2.
Figure 11.
committed by the Violation-Scopolamine-Saline group (see Figure 12). Both of these error types were absent on the last day of acquisition (see Figure 6, bottom panel).

**Acquisition of element types.** Figure 13 shows the mean percentage of chunk boundary element errors for all groups over the 14 days of acquisition of the unphrased pattern and drug transfer. Results showed an effect of drug transfer condition, with rats that received saline in transfer having the quickest overall rate of acquisition of the chunk boundary elements. A 2(Pattern) X 4(Drug) X 14(Day) mixed design ANOVA was conducted on rats’ daily mean percentage of chunk boundary element errors. Mauchly’s test indicated that the assumption of sphericity had been violated (chi-square = 297.42), therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (epsilon = 0.44). Main effects were found for drug, $F(3, 39) = 16.93$, $\eta_p^2 = 0.57$, and day, $F(5.72, 223.22) = 28.68$, $\eta_p^2 = 0.42$, and the Drug X Day interaction was significant, $F(17.17, 223.22) = 4.78$, $\eta_p^2 = 0.27$.

Planned comparisons showed that rats that received saline in acquisition and were transferred to scopolamine committed a significantly higher percentage of chunk boundary element errors Days 1-14 (see Figure 13, top panel). Planned comparisons also showed that rats that received scopolamine in acquisition and remained on scopolamine in transfer committed a higher percentage of chunk boundary element errors than rats that were transferred to saline on Days 7-14 (see Figure 13, bottom panel).

Figure 14 shows the mean percentage of within-chunk element errors for all groups over the 14 days of acquisition of the unphrased pattern and drug transfer. Results showed an effect of drug transfer condition, with rats that received saline in transfer
Figure 12: Mean percentage of error types at violation elements for Violation-Saline-Saline, Violation-Saline-Scopolamine, Violation-Scopolamine-Scopolamine, and Violation-Scopolamine-Saline groups. Percentages of errors were averaged across the violation elements for the 10 patterns on the first day of transfer of Experiment 2.
Figure 12.
Figure 13: *Top panel:* Acquisition curves for chunk boundary elements for Perfect-Saline-Saline, Perfect-Saline-Scopolamine, Violation-Saline-Saline, and Violation-Saline-Scopolamine groups over the 14 days of acquisition in Experiment 2. Daily mean percentage of errors was averaged across all chunk boundary elements of the patterns. *Bottom panel:* Acquisition curves for chunk boundary elements for Perfect-Scopolamine-Scopolamine, Perfect-Scopolamine-Saline, Violation-Scopolamine-Scopolamine, and Violation-Scopolamine-Saline groups over the 14 days of acquisition in Experiment 2. Daily mean percentage of errors was averaged across all chunk boundary elements of the patterns. Error bars: ± SEM. Closed symbols indicate rats stayed on same drug as in acquisition; open symbols indicate rats transferred to different drug.
Figure 13.
having the quickest overall rate of acquisition of the unphrased pattern within-chunk elements. A 2(Pattern) X 4(Drug) X 14(Day) mixed design ANOVA was conducted on rats’ daily mean percentage of within-chunk element errors. Mauchly’s test indicated that the assumption of sphericity had been violated (chi-square = 278.88), therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (epsilon = 0.43). Main effects were found for drug, $F(3, 39) = 16.19$, $\eta_p^2 = 0.56$, and day, $F(5.59, 217.81) = 13.39$, $\eta_p^2 = 0.26$, and the Drug X Day interaction was significant, $F(16.76, 217.81) = 3.81$, $\eta_p^2 = 0.23$. Planned comparisons showed that rats that received saline in acquisition and were transferred to scopolamine committed a significantly higher percentage of within-chunk element errors Days 1-8 and 10-14 (see Figure 14, top panel). There were no consistent differences between rats that received scopolamine in acquisition and either drug transfer condition (see Figure 14, bottom panel).

Figure 15 shows the mean percentage of violation element errors for the Violation pattern groups over the 14 days of acquisition of the unphrased pattern and drug transfer. Results showed an effect of drug transfer condition, with rats that received saline in both acquisition and transfer showing the quickest rate of acquisition of the violation element. A 2(Pattern) X 14(Day) repeated measures ANOVA was conducted on rats’ daily mean percentage of violation element errors. Mauchly’s test indicated that the assumption of sphericity had been violated (chi-square = 124.36), therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (epsilon = 0.47). A main effect was found for drug, $F(3, 20) = 4.97$, $\eta_p^2 = 0.43$, but no other main effects or interactions were significant.
Figure 14: Top panel: Acquisition curves for within-chunk elements for Perfect-Saline-Saline, Perfect-Saline-Scopolamine, Violation-Saline-Saline, and Violation-Saline-Scopolamine groups over the 14 days of acquisition in Experiment 2. Daily mean percentage of errors was averaged across all within-chunk elements of the patterns. Bottom panel: Acquisition curves for within-chunk elements for Perfect-Scopolamine-Scopolamine, Perfect-Scopolamine-Saline, Violation-Scopolamine-Scopolamine, and Violation-Scopolamine-Saline groups over the 14 days of acquisition in Experiment 2. Daily mean percentage of errors was averaged across all within-chunk elements of the patterns. Error bars: ± SEM. Closed symbols indicate rats stayed on same drug as in acquisition; open symbols indicate rats transferred to different drug.
Figure 14.

- Perfect-Saline-Saline
- Perfect-Saline-Scopolamine
- Violation-Saline-Saline
- Violation-Saline-Scopolamine

- Perfect-Scopolamine-Scopolamine
- Perfect-Scopolamine-Saline
- Violation-Scopolamine-Scopolamine
- Violation-Scopolamine-Saline
Figure 15: Acquisition curves for violation element for Violation-Saline-Saline, Violation-Saline-Scopolamine, Violation-Scopolamine-Scopolamine, and Violation-Scopolamine-Saline groups over the 14 days of acquisition in Experiment 2. Error bars: ± SEM. Closed symbols indicate rats stayed on same drug as in acquisition; open symbols indicate rats transferred to different drug.
Figure 15.

![Graph showing mean violation element errors over days for different conditions (Violation-Saline-Saline, Violation-Saline-Scopolamine, Violation-Scopolamine-Scopolamine, Violation-Scopolamine-Saline). The y-axis represents the mean % violation element errors, and the x-axis represents the days from 1 to 14. The graph demonstrates the trend and variability of violation errors across different conditions and days.]
Discussion

Experiment 2 produced additional evidence for the role of the cholinergic system in sequential learning, particularly in the initial encoding of phrasing cues and of strategy development in the absence of those cues. In examining the overall effect of the phrasing cue removal and drug transfer on Day 1 of Experiment 2, we observed that if rats are trained to a high criterion of performance under saline and then transferred to a similar unphrased pattern, the presence of a violation element makes the rats more sensitive to the phrasing cue removal, as revealed by the overall error analysis. Further, the impairment observed in both pattern groups for rats that are transferred from saline to scopolamine with the phrasing cue removal suggests that the addition of scopolamine significantly increases rats’ sensitivity to the phrasing cue removal. On the other hand, we also observed that rats that were trained on scopolamine and then transferred to saline showed a similar increase in overall errors, suggesting the possibility of a state-dependent effect in both different drug transfers (Saline-Scopolamine and Scopolamine-Saline).

Previous literature suggests that scopolamine and similar drugs are capable of producing state-dependency (Berger & Stein, 1969; Costa & Xavier, 2007; Petersen, 1979). Costa and Xavier (2007) demonstrated that atropine causes rats to encode information differently in training than rats that are given saline, and for rats trained under saline, atropine can also disrupt retrieval of learned information; it is only when drug states are congruent, training to test, that some savings may occur for rats given atropine. However, it should be noted that this disruption in retrieval was task-dependent,
in that retrieval of spatial visual information is more susceptible to disruption by atropine than non-spatial.

Berger and Stein (1969) found evidence for an “asymmetrical dissociation”, meaning that deficits caused by training under one drug condition (i.e., scopolamine) and transferring to the other drug condition (i.e., saline) cannot be accounted for by encoding failure during acquisition or a state-dependent effect alone, but rather the two working in combination. In their study that examined the possibility for state-dependent learning with scopolamine on a conditioned drink suppression task, they found that rats that received scopolamine during training and were then tested under saline showed the worst performance, compared to saline-saline, saline-scopolamine, and scopolamine-scopolamine groups (Berger & Stein, 1969). This suggests that scopolamine interfered with acquisition, but a change in drug state further dissociated learning by disrupting internal retrieval cues.

We cannot rule out that a similar asymmetrical dissociation may be taking place in analysis of Day 1 of transfer compared to Day 49 of acquisition, given the increase in groups that were trained on saline or scopolamine and transferred to scopolamine, as well as groups that were trained on scopolamine and transferred to saline, while rats that were trained on saline and remained on saline maintaining good performance in transfer. However, the state-dependent argument is weakened by examining the 14 days of acquisition of the unphrased pattern under the drug transfer conditions which reveals that rats in the Scopolamine-Saline and Saline-Scopolamine groups were performing fewer overall errors by Day 14 than rats in the Scopolamine-Scopolamine groups. This
evidence also weakens the possible argument of tolerance effects, as the Scopolamine-Scopolamine group maintained an asymptotic level of performance throughout the transfer to the unphrased pattern.

These results are also in line with Ragozzino and Choi (2004). Recall that they found an increase in acetylcholine output in the medial striatum during reversal learning; thus, a cholinergic blockade, such as scopolamine, would interfere with learning a reversal task. Experiment 2 provided evidence that scopolamine did cause an initial impairment of acquisition in transfer, but rats with prior learning to a high criterion under saline were able to overcome this scopolamine-induced deficit in performance.

The results of Experiment 2 also support an experiment by Whishaw (1985) in which atropine-treated and saline-treated rats were trained on a place navigation task in a Morris water maze. Rats were then transferred to a drug reversal in which the atropine-treated rats were tested on saline and saline-treated rats were tested on atropine. Whishaw (1985) found that rats that were at first trained on saline and transferred to atropine showed greater initial impairment in the transfer than rats that were trained on atropine and transferred to saline. Following several blocks of drug reversal training, the platform location was repositioned and both groups showed severe impairment. An additional experiment by Whishaw (1989) examined the effects of a drug and task transfer in rats. In this experiment saline-rats were trained on a place navigation task in a Morris water maze in one room and were then moved to a second room where rats learned a new place task while transferred to either saline or atropine. Rats that remained on saline showed some initial impairment when trained in the second room on the new task, but returned to the
same level of performance as demonstrated in the first room after three days of training. Rats that transferred to atropine, however, showed even greater impairment in the second room and did not return to the same level of performance as in the first room by the end of five days of training.

Based on what Whishaw found, it was expected in Experiment 2 that rats given scopolamine in transfer would be more impaired than rats given saline, with rats trained on scopolamine and transferred to scopolamine most likely showing the greatest impairment. It was also expected that rats trained on scopolamine and transferred to saline would show some initial impairment but would learn their respective patterns at a faster rate of acquisition than rats given scopolamine in transfer. This hypothesis was supported in that rats in the Saline-Scopolamine conditions were more impaired in their performance on the first day of transfer than rats in the Saline-Saline conditions, and rats in the Scopolamine-Saline conditions also showed this increased impairment. Further, by the end of acquisition of the unphrased pattern, with rats in the Scopolamine-Scopolamine condition maintaining their impaired performance at an asymptotic level at a higher rate of errors than the other groups.

It was also hypothesized that rats in the violation pattern condition would show more overall impairment than rats in the perfect pattern condition in transfer. This hypothesis was partially supported when examining overall errors; however, when examining errors by element type, rats in the Perfect-Saline-Scopolamine and Perfect-Scopolamine-Scopolamine groups showed more impairment in acquisition of the unphrased pattern on chunk boundary elements and the Perfect-Scopolamine-
Scopolamine group also showed greater impairment on within-chunk elements, relative to their Violation pattern counterparts, though it should be noted that there was only a trend for differences between pattern types and these differences were not statistically significant. Nevertheless, this indicates that the increased overall impairment observed in these Violation pattern groups was due to an increase in violation element errors.

Finally, a hypothesis based on what was found in Chenoweth (2007) suggested that scopolamine would interfere with rats’ abilities to develop strategies to make the correct responses in the absence of temporal cues. The comparison of most interest was between rats that stayed in the same drug condition (i.e., Scopolamine-Scopolamine, Saline-Saline) versus rats that transferred to a different drug condition (i.e., Scopolamine-Saline, Saline-Scopolamine). The error analyses of the first day of transfer compared to the last day of acquisition of Experiment 1 indicated that this hypothesis was not supported. While there were some differences in types of errors committed, we did not observe the same breakdown in strategy development in rats given scopolamine in Experiment 2 as rats that were given atropine in Chenoweth (2007). In all groups, rats were able to narrow their responses to within one or two receptacles surrounding the correct receptacle.
General Discussion

The present study provided a unique opportunity to investigate the effects of the muscarinic cholinergic blockade by scopolamine in acquisition of a perfect versus a violation pattern, as well as the effects in strategy development when temporal cues were removed. In Experiment 1, we observed that rats given scopolamine were impaired in their overall performance relative to rats given saline, but we saw only a weak transient interaction for pattern type. Additionally, the error analyses yielded a similar pattern of errors for Perfect-Scopolamine and Violation-Scopolamine groups. This indicates that the addition of a violation element does not interact significantly with the presence of scopolamine to produce impairments in pattern acquisition beyond what is necessary to learn about chunk boundary and within-chunk elements.

Experiment 2 extended this investigation to examine if the observed differential impairments from initial acquisition affected performance in task and drug reversal learning. It was evident that distinctly different cognitive processes must be involved in the acquisition of the element types, as scopolamine dissociated chunk boundary versus violation element cognitive systems. This was made clear on the first day of transfer, as a large effect of scopolamine was observed at chunk boundary elements when cues were removed. That is, a significant increase in chunk boundary element errors was observed in rats that received scopolamine in acquisition, transfer, or both acquisition and transfer, relative to those who received saline in both acquisition and transfer (see Figure 7). This suggests that being exposed to scopolamine during encoding, retrieval, or both, disrupts
rats’ abilities to make correct responses at chunk boundary elements in the absence of
temporal cues, indicating that central cholinergic systems are crucial for encoding and
retrieving the chunk boundary response. This evidence is counter to that observed in the
violation element in that on the first day of transfer there was no additional impairment of
violation element performance with the addition of scopolamine. That is, a significant
increase in violation element errors was observed in rats that received saline in
acquisition regardless of whether they received saline or scopolamine when transferred to
the unphrased pattern (see Figure 9). This suggests that while exposure to scopolamine
appears to disrupt acquisition and retention of the violation element response, this effect
may be a side effect of the impairment observed at chunk boundary elements. Thus, while
central cholinergic systems appear to be necessary for making correct responses at the
violation element, it is only because they are necessary for proper encoding and retrieval
of the chunk boundary element response. This suggests that there are different cognitive
systems that underlie performance of the chunk boundary elements than those that
underlie the violation element. Thus, the differences between the drug reversal conditions
were not as great as would have been observed if the same processes were being
recruited. Furthermore, examination of within-chunk element performance on the first
day of transfer revealed only a weak effect of scopolamine (see Figure 8), not unlike that
observed in prior studies using MK-801 and atropine, both of which produced the similar
strong effects at the chunk boundary and violation elements observed in the present study
(Chenoweth, 2007; Fountain & Rowan, 2000). In short, these results provide evidence
that parallel processing is taking place for the acquisition of chunk boundary, within-
chunk, and violation elements concurrently. The next step is to determine which structures are involved in this parallel processing.

Some structures that have been explored within the context of sequential learning have been the hippocampus and basal ganglia (Fountain, 2006), though lesions to these specific structures have only shown a mild deficit compared to the severe deficits in acquisition as a systemic blockade of muscarinic receptors. This suggests that while these individual structures may be involved, perhaps it is the communication between structures within the brain that allows for the acquisition of these different element types. A systemic muscarinic cholinergic antagonist blocks these pathways, effectively “turning off” the communication between these structures. What are unknown are the specific pathways involved in learning these different element types. Experiment 1 gives support to the idea that the same muscarinic cholinergic pathway or pathways are utilized to learn the different elements of the sequential pattern in that chunk boundary elements were unaffected by the presence of a violation element. Again, this suggests that parallel processing is involved in learning about these elements. A key question following the results of the present study would be to investigate the extent to which these two systems overlap.

It has been suggested by this and other studies by Fountain and colleagues (Fountain & Benson, 2006; Fountain, 2006; Fountain et al., 2007) that multiple memory processes are recruited in learning sequential patterns under normal, drug-free conditions. Fountain and Benson (2006) demonstrated that rats use chunking, rule learning, and interitem association learning in parallel to learn complex sequential patterns. In that
study, rats learned one of three interleaved patterns: a Structured/Structured (S-S) Pattern (1526374851627384), a Two Violation/Structured (2V-S) Pattern (1526473851627384), or a Four Violation/Structured (4V-S) Pattern (1526473861527384). Rats learned the S-S pattern more quickly than the 2V-S and 4V-S patterns. Furthermore, rats learned the first sub-patterns more quickly than the second subpattern, indicating that the rats had separated the two patterns into “chunks”.

Additional analyses of the types of errors made for each sub-pattern revealed some interesting differences. In the second sub-pattern (same for all three groups: 56781234), there was evidence that rats were using multiple-item associations to make responses. For example, when cues present the same outcomes, learning is easier (e.g., in the 4V-S Pattern, 1-5 predicts 2). Likewise, when cues present different outcomes, learning is more difficult (e.g., in 4V-S Pattern, 1-5-2 predicts 6 and 7). In the first sub-pattern, on the other hand, there is clear evidence for the use of rule-based responding. For example, the 2V-S and 4V-S Pattern groups applied the same rule (i.e., go one lever to the right), despite the fact that they were never reinforced for that two-item association at any other point in the pattern:

2V-S Pattern: 1 5 2 6 4 7 3 8 5 1 6 2 7 3 8 4
4V-S Pattern: 1 5 2 6 4 7 3 8 6 1 5 2 7 3 8 4
Error Response: 3 5 4 7

This clear demonstration of different forms of learning employed concurrently to acquire a sequential pattern may also account for the dissociations in learning of different
element types (e.g., within chunk versus chunk boundary elements) in the presence of a muscarinic cholinergic antagonist, such as scopolamine. Further, the evidence from Chenoweth (2007) suggested that the type of learning involved in acquiring correct within chunk element responses is insensitive to cholinergic manipulations. In contrast, the present study did show a slight but significant impairment of within-chunk elements. In a direct comparison the Violation-Scopolamine group to the results of the atropine-treated rats in the prior study by Chenoweth (2007), one might argue that rats in the atropine study may have learned more about the sequential pattern than rats in the present study. However, the Violation-Scopolamine rats in the present study showed less overall impairment in acquiring the chunk boundary and violation elements than the atropine-treated rats. Thus, the differences between the two studies may be attributed more to a difference in drug dose, not in qualitatively different effects of drugs or reinforcers.

The question remains of how do we explain this dissociation between the three pattern elements, in particular the dissociations we see in Experiment 2 when rats are transferred to an unphrased pattern? There are several possible explanations; we will explore the following three: methodology, timing, and attention.

First, there may be some differences in methodology. It was noted in the present study using the water chamber procedure that the phrasing cue removal did not appear to have the same level of effects on control animals as what we have observed previously in the lever chamber procedure. One possible difference is the salience of the phrasing cue. In the lever chamber procedure, retractable levers are entering and exiting the chamber, creating a considerable amount of noise, as well as the physical presence and absence of
the levers. Thus, when levers are not moving for the 3-s phrasing cue, it is more noticeable than the absence of lights in the back of nose-poke receptacles. This difference in salience may have caused the rats to encode the patterns differently in the water chamber procedure than in the lever chamber procedure. Further, rats given scopolamine might be more sensitive to these changes in cues than those on saline when it comes to chunk boundary performance.

A second explanation that may hold more weight for the disruptions we observed at chunk boundary elements as well as the violation element is timing. By removing the phrasing cues in the transfer pattern in Experiment 2, we effectively changed the timing and pace of the pattern that these rats had spent 49 days learning in Experiment 1. In a study by Meck (1983), it was found that the addition of the a muscarinic cholinergic blockade atropine decreased memory storage processing speed. In this experiment, rats were trained on a temporal discrimination procedure in which two time intervals were reinforced, 2-s and 8-s, following a noise signal. Rats were given physostigmine, an acetylcholinesterase inhibitor, atropine, a cholinergic antagonist, or saline. Rats given saline were able to accurately distinguish between the two temporal durations, while physostigmine-treated rats showed a leftward shift in time assessment and atropine-treated rats showed a rightward shift in time assessment. In short, this means that while both drug groups learned to make the correct response, physostigmine-treated rats encoded the temporal intervals as “shorter” than they really were, i.e., an “underestimation”, indicating an increase in memory storage processing speed, while atropine-treated rats encoded the temporal intervals as “longer” than they really were, i.e.,
and “overestimation”, indicating a decrease in memory storage processing speed. Meck and colleagues have supported a model that implicates the role of cholinergic systems located in the frontal cortex and the medial temporal lobe as part of a larger neural network involved in interval timing (Buhusi & Meck, 2005; Meck, 1996; 2005). In this model, acetylcholine is necessary for the correct updating of reference memory as informed by working memory. If a cholinergic blockade is present, this update system is slowed down, creating an incorrect overestimate of interval timing (Buhusi & Meck, 2005).

An application of these results and proposed timing model to the present study would suggest that rats trained on scopolamine may have encoded the correct response at the phrasing cue, but may have encoded the duration incorrectly as being longer than 3-s. When those phrasing cues were then removed in Experiment 2, rats that were trained on scopolamine in Experiment 1 and were then transferred to scopolamine were unable to make an appropriate adjustment in the timing of the pattern. Additionally, rats that were trained on scopolamine in Experiment 1 and transferred to saline in Experiment 2 showed an initial impairment due to a holdover of the incorrectly encoded timing of the pattern under scopolamine in Experiment 1, but were able to overcome that incorrect encoding to learn the new timing of the pattern in Experiment 2. Likewise, we observed relatively little impairment in the Saline-Saline transfer, indicating that rats were able to make an appropriate adjustment of timing in Experiment 2; but rats that were in the Saline-Scopolamine transfer showed difficulty in making this adjustment, indicating that
scopolamine interfered with rats’ abilities to adjust the timing of correct responses in the transfer from a phrased pattern to an unphrased pattern.

A third possible explanation for the observed dissociations is that we are disrupting rats’ abilities to attend to pattern elements, namely where they occur. One could argue that this may be what is happening in with the scopolamine-treated rats at the chunk boundary elements when the phrasing cues are removed. The saline-treated rats are able to overcome the timing issues to show only a slight impairment at the chunk boundaries that quickly goes away in acquisition of the unphrased pattern, but under the influence of scopolamine, perhaps an inability to attend to where they are in the chamber at these parts in the pattern and without the presence of the phrasing cues can explain this additional impairment.

**Conclusion.** As stated previously, the present study provided a unique opportunity to investigate the effects of the muscarinic cholinergic blockade by scopolamine in acquisition of a perfect versus a violation pattern, as well as the effects in strategy development when temporal cues were removed. There are numerous hypothesized mechanisms that appear to be employed in the acquisition and retention of sequential patterns of behavior, and the present study adds to this literature with results demonstrating the dissociation by cholinergic blockade of separate cognitive systems working in parallel to acquire and retain sequential patterns. Further, this study complements findings in the area of motor-skill learning, particularly the neuropsychological theory of motor-skill learning developed by Willingham (1998; 1999). His theory implicates the neural structures discussed above, namely basal ganglia,
as playing key roles in the sequencing process that supports motor-skill learning, citing
deficits in sequential performance in patients with Huntington’s disease and Parkinson’s
disease that show basal ganglia damage (Willingham, 1998; 1999). An area of
Willingham’s motor-skill learning theory that is lacking in evidence, however, is the
determination of how exactly sequences are learned (Willingham, 1998). A related theory
on the neurobiology of skill learning proposed by Salmon and Butters (1995) suggests
that the cerebellum may play a partial role in sequencing behavior by organizing
sequential motor movements according to their timing. This timing information is then
sent to the basal ganglia for further consolidation as a set “motor program” underlying the
observed motor behavior. The present study strongly suggests that the cholinergic system
is crucial for encoding and retrieving some aspects of sequential learning, and both the
cerebellum and basal ganglia contain muscarinic cholinergic receptors (Ehlert, Roeske, &
Yamamura, 1995). Perhaps the disruptions we observed in chunk boundary element
performance, specifically those seen in the phrasing cue removal transfer, are a result of
the disruption in the proposed “timing message” from the cerebellum to the basal ganglia.
However, additional research needs to be conducted on underlying mechanisms of the
multiple systems involved in sequential learning to further characterize the nature of how
we organize and use this type of complex information for planning and anticipating
events in our everyday lives.
References


