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SERIAL PATTERN RULE EXTRAPOLATION IS SPARED DURING A MUSCARINIC CHOLINERGIC CHALLENGE IN RATS (59 pp.)

Thesis Advisor: Stephen B. Fountain

Rats have the capacity to extrapolate a known sequence of events to anticipate a novel item. We examined whether or not rats can extrapolate a serial pattern during a muscarinic cholinergic challenge. Adult male and female rats learned to nosepoke a sequential pattern of responses in a circular array of 8 receptacles attached one each to the walls of an octagonal chamber. This training pattern consisted of seven 3-element chunks of a rule-based serial pattern, namely, 123-234-345-456-567-678-781. On the day after meeting a high criterion on the training pattern, rats were given i.p. injections of 0.6 mg/kg scopolamine hydrobromide, a muscarinic cholinergic blocker, before encountering patterns consisting of the 7-chunk training pattern plus an added eighth chunk. The added chunk was either consistent with pattern structure (chunk "812") or contained a terminal element that violated pattern structure (chunk "818", where the violation element is underlined). Under scopolamine, and even while showing scopolamine-induced impairments of performance throughout the pattern, rats in both groups extrapolated known pattern structure in the novel added chunk, producing approximately 60% rule-consistent "2" responses on the terminal element of both types of chunks. Thus, despite scopolamine exposure, both male and female rats extrapolated well-learned pattern structure to a new chunk. Whereas earlier work showed that muscarinic cholinergic suppression had little effect on rule learning during acquisition of a pattern, the current study demonstrated that intact muscarinic cholinergic neurotransmission is not necessary for extrapolation of a well-learned rule to a novel chunk.

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By

Megan E. Miller-Cahill

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Thesis written by

Megan E. Miller-Cahill

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Approved by

Stephen B. Fountain, Ph.D, Advisor, Department of Psychological Sciences

Maria Zaragoza, Ph.D, Chair, Department of Psychological Sciences

James L. Blank, Ph.D, Dean, College of Arts and Science

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Acknowledgments

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Serial Pattern Rule Extrapolation is Spared During a Muscarinic Cholinergic Challenge in Rats

Quite a number of studies have demonstrated that nonhuman animals are sensitive to the structure of serial patterns, beginning with the work on rat serial pattern learning by Hulse and his colleagues (e.g., Hulse & Dorsky, 1977, 1979; Fountain & Hulse, 1981). By training rats in runways for sequences of different food pellet quantities, Hulse & Dorsky (1977, 1979) demonstrated that rats learned faster to anticipate food quantities that followed consistent rules compared to patterns that did not. For example, rats trained on the sequence "14-7-3-1-0" learned to anticipate the next quantity of food pellets throughout the pattern by slowing run times for each successive quantity. The reasoning was that the food pellet quantities in the structured pattern followed a simple "less than" rule that allowed rats to anticipate what the next quantity should be. Hulse and Dorsky (1977) showed that rats in the structured pattern ("14-7-3-1-0") group ran significantly slower to the "0" pellet quantity compared to rats in the unstructured pattern ("14-1-3-7-0") group, consistent with the view that rats learned rules to encode pattern structure (Hulse & Dorsky, 1977).

In a second experiment, Hulse & Dorsky (1977) showed that a strongly structured pattern of food quantities of "14-7-3-1-0" allowed for better rule utilization than a weakly structured pattern of "14-5-5-1-0". Overall, these two experiments demonstrated that rule-learning theory predicted pattern difficulty in rats. In a follow-up set of studies, Hulse & Dorsky (1979)

demonstrated rule generalization, that is, that rats could transfer a rule learned in one pattern of food quantities to facilitate learning a novel pattern if the pattern followed the same rule. Finally, Fountain & Hulse (1981) showed that rats could extrapolate a rule from a subset of food quantities to anticipate a novel element added to a trained pattern. Rats in the Fountain & Hulse (1981) experiment were trained on one of three 4-element patterns, then they were transferred to the same patterns with an added "0" pellet quantity at the end or a one-day test for rule extrapolation. Despite having no previous experience with the novel added quantity, rats trained on a rule were able to extrapolate that rule to make a rule-consistent response on a novel element (Fountain & Hulse, 1981).

The foregoing early experiments using runways and simpler serial patterns demonstrated that rats are capable of learning rules describing pattern structure, generalizing these rules to a novel pattern with similar structure, and extrapolating a pattern to predict a novel element (Hulse & Dorsky, 1977, 1979; Fountain & Hulse, 1981). Comparable phenomena have been observed in a more complex learning and memory paradigm that bears more similarity to human sequential learning paradigms. The serial multiple choice (SMC) task was developed by Fountain & Rowan (1995a; 1995b) as an adaptation of a nonverbal serial pattern learning task designed by Restle & Brown (1970) and adapted for rats by Fountain, Raffaele, & Annau (1986). The SMC task can be used to assess complex forms of serial pattern learning in rats which parallel those observed in mice, pigeons, and humans (Garlick, Fountain, & Blaisdell, 2016; Kundey et al., 2013; Rowan, Fountain, Kundey, & Miner, 2001). Serial pattern learning by rats in the SMC task involves using multiple cognitive processes concurrently (Muller & Fountain, 2010, 2016).

The SMC task is a discrete-trial procedure in an octagonal operant chamber. On each wall of the chamber is a receptacle, giving rats 8 choices on each trial. If the rat makes a correct

response, the rat is reinforced and can continue to the next trial. If the rat makes an incorrect response, the rat undergoes a correction procedure. For the correction procedure, all of the receptacle lights in the chamber extinguish except for the correct receptacle. Rats are forced to nosepoke the correct receptacle, at which all of the receptacle lights come back on and the trial can continue. Rats are trained on a highly structured training pattern such as:

123-**2**34-**3**45-**4**56-**5**67-**6**78-**7**81-**8**12-…(repeat)

This pattern is highly structured. Previous research has demonstrated that elements at the beginning of these chunks, termed "chunk-boundary" elements (shown in bold font in the training pattern above) are learned differently than elements within each chunk, termed "within-chunk" elements (e.g., Fountain, 1995b; Kundey & Fountain, 2014; Muller & Fountain, 2010). For chunk-boundary elements, the phrasing cue along with chunk length and timing cues serve as discriminative cues signaling to turn back one receptacle. This is a form of stimulus-response (S-R) learning where the stimulus is the phrasing cue (Fountain, 1995b, Rowan, & Wollan 2013; Stempowski, Carman, & Fountain, 1999). The phrasing cues serve to break the pattern into seven chunks. For within-chunk elements, rats learn to turn forward one receptacle (a "clockwise 1" or "+1" rule) until a 3-sec phrasing cue occurs. This is a form of abstract rule learning. The rule-learning strategy rats use is to learn to move "+1" receptacle from their current receptacle position in the chamber (Fountain, 1995b; Muller & Fountain, 2010, 2016). Highly structured elements described by simple repeating rules are easy to learn and facilitate acquisition, whereas items that do not fit the structure are difficult to learn and will slow acquisition (Hulse, 1984; Fountain et al., 1984; Fountain, 1990).

Acetylcholine (ACh) neurotransmission is important for many learning and memory processes (Gold, 2003; Hasselmo, 2006). Acetylcholine binds to two types of cholinergic

receptors, nicotinic and muscarinic (Gotti, Zoli, & Clementi, 2006; Langmead, Watson, & Reavill, 2008; Levin, 1992). Cholinergic pathways project through the brain, including the frontal cortex, hippocampus, basal ganglia, and amygdala, all of which are areas known to be of importance to learning and memory processes (Mesulam et al., 1983; Selden et al., 1998). Activation of nicotinic or muscarinic acetylcholine receptors has been shown to facilitate learning. In contrast, deactivation of nicotinic or muscarinic receptors can impair learning (Hasselmo, 2006). Since the 1960's, researchers have used scopolamine as an amnestic agent in studies of memory (Bohdanecký & Jarvik, 1967). Since then, it has been established that the administration of scopolamine prior to training produces pronounced cognitive deficits at testing for multiple types of tasks. Specifically, studies have assessed the muscarinic cholinergic suppression of associative learning, declarative memory, discrimination learning, spatial memory, fear memory, and serial learning (Ebert & Kirch, 1998; Klinkenberg & Blokland, 2010; Nissen, Knopman, & Schacter, 1987; Petersen, 1977; Whishaw, 1989; Wesnes, Simpson, & Kidd, 1988). In contrast, scopolamine administration prior to training does not impair implicit learning, procedural memory, or reference memory (Beatty & Bierley, 1985; Nissen, Knopman, & Schacter, 1987).

Previous research using the SMC task has assessed whether muscarinic cholinergic neurotransmission is necessary for serial pattern acquisition. Results showed that studying serial pattern learning under cholinergic suppression can be a useful tool in dissociating the learning mechanisms associated with specific element types (Chenoweth & Fountain, 2015; Fountain, Rowan, & Wollan, 2013; Muller & Fountain, 2014;). Using the SMC task, Fountain, Rowan, & Wollan (2013) assessed the effects of atropine sulfate (hereafter atropine), a muscarinic acetylcholine antagonist, on performance of well-learned serial patterns. Rats learned either a

highly-structure serial pattern or a pattern with one element at the end of the pattern (the "terminal element") that violated pattern structure. Once trained to a high criterion of less than 10% errors on all elements of the pattern, rats were given an intraperitoneal (i.p.) injection of 50mg/kg atropine prior to testing on the training pattern. Results of the study showed a dissociation in the learning that occurred under muscarinic cholinergic suppression based on elemental type. Atropine caused very high error rates on the violation element, intermediate error rates for chunk-boundary elements, but no effect on within-chunk element performance.

The day after testing with atropine, Fountain and colleagues (2013) gave rats in both pattern groups an injection of saline and tested them on the training patterns. Performance across all elements in both pattern groups was not significantly different from performance on criterion day when errors on all elements were at or below 10%. These results show that atropine impairment on a previously learned serial pattern were not permanent and that muscarinic cholinergic function is differentially important for performance of different element types. Atropine methyl nitrate (AMN), a form of atropine that does not readily cross the blood-brain barrier, produced none of these effects (Fountain et al., 2013). Because atropine sulfate caused deficits that were not observed when rats were exposed to AMN, it was concluded that the deficits caused by atropine sulfate were due to central muscarinic suppression not peripheral muscarinic suppression.

In a follow-up to the Fountain, Rowan, & Wollan (2013) experiment, Chenoweth & Fountain (2015) assessed learning of the same serial patterns used by Fountain, Rowan, & Wollan (2013) with daily administration of atropine or saline during serial pattern training. Daily administration of 50 mg/kg atropine prior to daily training caused moderate impairment of chunk-boundary element learning with asymptote at approximately 50% errors. Thus,

discrimination learning mechanisms were partially impaired. Violation element learning was severely impaired; rats never showed any learning. In contrast, within-chunk element learning was not impaired compared to saline controls, showing that rule learning mechanisms were not affected by atropine. Both Fountain, Rowan, & Wollan (2013) and Chenoweth & Fountain (2015) analyzed the types of errors rats made and found that on the violation element "8" in the chunk "818" rats made mostly "2" responses ("812"), that is, a rule-consistent response.

Although we know that rats are sensitive to pattern structure and are able to use rule-based learning mechanisms under muscarinic cholinergic suppression, it is unknown whether or not rats would be able to extrapolate previously-learned rules while under muscarinic cholinergic suppression. Fountain & Rowan (2000) provide a potential model for such an experiment. In order for rats to extrapolate a learned rule to a novel sequence, rats would have to demonstrate the ability to extrapolate rules learned in a previously learned chunk of information to a novel chunk of information. Fountain & Rowan (2000), trained rats on a 21-element serial pattern (123-234-345-456-567-678-781) to a criterion of less than 10% errors on chunk-boundaries and within-chunk elements. The following day, rats were given MK-801, a NMDA antagonist, or saline as control and were tested on the training pattern with an added novel 3-element chunk. The novel added chunk was either structurally consistent with the training pattern (812) or contained one element that violated pattern structure (818). Results of this study demonstrated that adding elements to a pattern that are rule-consistent did not impair rat performance, but adding an element that violated pattern structure did impact chunk-boundary performance regardless of NMDA blockade. A more severe impairment was seen in chunk-boundary performance with NMDA blockade (Fountain & Rowan, 2000). Thus, Fountain & Rowan (2000) demonstrated that the SMC task could be used to assess rat rule extrapolation and application to

a novel chunk. Interestingly, saline injected controls that received a novel added chunk did not have an increase in within-chunk or chunk-boundary errors regardless of whether the added novel chunk was rule-consistent or had an element that violated pattern structure. Saline injected controls that received a novel chunk with a violation element did have high errors on the violation terminal element. Rats exposed to either MK-801 or saline that received a rule-consistent terminal element did not differ significantly in their responses in that errors were still under the 10% criterion for transfer. This shows that neither the addition of MK-801 or the addition of a novel chunk interfered with rat rule extrapolation (Fountain & Rowan, 2000).

Using the same design as Fountain & Rowan (2000), the current experiment sought to determine if rats could extrapolate rules about pattern structure to apply to a novel chunk during muscarinic cholinergic suppression. To assess rule extrapolation, rats were trained on the highly structured training pattern:

123-**2**34-**3**45-**4**56-**5**67-**6**78-**7**81… (repeat)

After reaching a criterion of 90% or more correct responses on all pattern elements for three consecutive days, rats were randomly assigned to one of two transfer chunk groups. For the drug transfer, five female rats and five male rats were randomly assigned to the Rule-Consistent group, and six female rats and six male rats were randomly assigned to the Violation group:

Group	**Training Pattern:**	**Novel Chunk:**
Rule-Consistent	123-234-345-456-567-678-781	812
Violation	123-234-345-456-567-678-781	818

The first day of the drug challenge regimen included giving rats a 1.0 ml/kg intraperitoneal (i.p.) vehicle injection of physiological saline (0.9%) 30 minutes before testing on the training pattern to ensure that rats were capable of maintaining the 90% or higher correct

response criterion despite being given i.p. injections. Rats took a maximum of two days to demonstrate maintenance of criterion. Following the day that maintenance of criterion was observed, rats were given an i.p. injection of 0.6 mg/kg scopolamine hydrobromide (hereafter scopolamine), a muscarinic acetylcholine antagonist, 30 minutes prior to testing. For testing on this day, male and female rats were randomly assigned to one of the two novel 3-element-chunk groups. The Rule-Consistent group received a novel chunk that followed the same rule as in acquisition, chunk "812", to create the new pattern "123-234-345-456-567-678-781-812". The novel chunk requires rats to utilize chunk-boundary and within-chunk rules learned during training and employ the same rules to make correct responses. The Violation group received the chunk "818", to create the new pattern "123-234-345-456-567-678-781-818". The violation chunk contained two out of the three rule-consistent elements in the novel rule-consistent chunk but the last element, termed the terminal element, was an element that was not consistent with the structure of the training pattern. Rats would not be able to learn a violation element in one day (Muller & Fountain, 2010). Therefore, the violation terminal element was used to assess the treatment of a random element that rats were not capable of learning and were not able to use what has already been learned about pattern structure. When rats are given a muscarinic anticholinergic drug after being trained to make less than 10% errors on a violation element, most of the errors rats make are rule-consistent responses. However, all previous research has assessed violation element retention and not exposure to a violation under cholinergic suppression. Because the goal of this study was to assess rule extrapolation under cholinergic suppression, the violation served as a control for an element that would not be reinforced if rats made a rule-consistent response.

Previous studies have shown that 1) learning within-chunk elements is unaffected by daily muscarinic cholinergic suppression before training, and 2) that after rats are trained to a high criterion on a serial pattern, muscarinic cholinergic suppression does not impair within-chunk performance (Chenoweth & Fountain, 2015; Fountain, Rowan, & Wollan, 2000). These results demonstrate that within-chunk performance, which relies on abstract rule learning and performance, is unaffected by muscarinic cholinergic suppression during either memory encoding or memory retrieval. Based on these findings, we predicted that scopolamine administration would not impair rule extrapolation. Therefore, we predicted that rats in the Rule-Consistent group should make significantly more correct responses on the rule-consistent terminal element than rats in the Violation group would make on the violation terminal element. A second indicator of rule extrapolation would be if rats performed similarly on the training pattern as on a novel added chunk. Despite a potential for scopolamine to increase errors on elements in the pattern, evidence from past research supports that rats should show intact abstract rule on the day of the scopolamine challenge (Chenoweth & Fountain, 2015). Therefore, if rule extrapolation is occurring, when we compare the terminal element of the training pattern (78**1** - shown in bold) to the terminal element of the rule-consistent chunk (81**2** - shown in bold), we should not find significant differences in correct responses.

Methods

Subjects

Twenty-two adult Long Evans rats (*rattus norvegicus*), eleven males and eleven females bred in-house, served as subjects for this experiment. For the duration of the experiment, rats were single-housed in plastic shoebox cages (40 cm wide x 85 cm long x 40 cm high) and given free access to food (LabDiet5P00 - ProLabRMH3000) and environmental enrichment in the form of paper towels and Nylabone chew toys. Rats were reinforced with water during shaping and behavioral testing and were restricted on water intake to 5 minutes of free access per day in addition to the water they received daily during testing. Rats were weighed weekly to ensure that all subjects were at or above 80% of free feeding weight despite being restricted of water intake. Rats were kept on a 15:9-h light-dark cycle that is standard for the facility.

Apparatus

For this experiment, we used three clear Plexiglas shaping chambers (15 cm wide x 30 cm long x 30 cm higher) that contained stainless steel wire mesh flooring and a single nose poke receptacle (2.5-cm diameter PVC pipe end caps painted flat black) that was centered on one end wall 5.cm above the floor. Each receptacle contained an infra-red emitter and detector located on the left and right sides as well as a white LED cue light positioned at the back of each receptacle. There were no other lights present during testing other than the LED cue lights used in the receptacles. The testing room light was turned off during testing.

Rats were trained in the serial multiple choice (SMC) task in six clear 1/4-inch Plexiglas octagonal test chambers (15 cm wide x 30 cm tall walls with 40 cm between opposite walls) with wire mesh floors. Each of the eight walls was equipped with a nosepoke receptacle (described above) centered 5.0 cm above the floor. Each nosepoke receptacle was connected at the bottom to a solenoid and syringe by plastic Tygon tubing, which served to deliver water reinforcement to the chamber. All chambers were enclosed within sound attenuating chambers with 10-ml syringes attached to an internal wall of the enclosure that served as water reservoirs. Syringes were connected by the Tygon tubing (VWR Scientific, Performance Plastics 1/32-inch, #R-3603) to solenoids (General Valve Corp. Vac. 20 psig. 24 V) and then to the nosepoke receptacles. Solenoids controlled the delivery of the water droplets to the nosepoke receptacles. As with shaping, there were no other lights present during testing other than the LED lights used in the receptacles. The testing room light was turned off during testing. See Figure 1 for octagonal testing chamber picture.

Drugs

Scopolamine hydrobromide was administered at a dosage of 0.6 mg/kg. Scopolamine (Sigma) was dissolved in physiological saline in a volume of 1.0 ml/kg. Rats received saline an equivalent injection volume to scopolamine (1.0 ml/kg). All injections were given intraperitoneal (i.p.) 30 minutes prior to testing the day after criterion was reached.

Procedure

Rats were weighed to document free feeding weight and then removed from *ad libitum* water for 36 hours prior to shaping. Rats were shaped for two consecutive days to nosepoke for water reinforcement. On each trial, the receptacle light was illuminated and after each nose-poke response, rats were reinforced with a 0.025-ml droplet of water that was delivered through the

bottom of the receptacle via Tygon tubing from a water reservoir. After each reinforcement the receptacle light was extinguished for the intertrial interval of 1 second on day one of shaping and 2 seconds on day two of shaping. In order to complete the shaping procedure, rats made 240 nosepoke responses on both days of shaping to simulate the amount of responses needed to complete a 24-element serial pattern at 10 patterns per day. After shaping, all rats received five minutes of supplemental water in their home cages.

Rats were trained in the serial multiple choice (SMC) task in octagonal operant chambers the following day after the two-day shaping (see Figure 1 for a diagram of the chamber). At the beginning of each trial, all 8 nosepoke receptacles were illuminated and the rat was allowed to make a response at one of the eight receptacles. As shown in Figure 1, the eight receptacles are arbitrarily numbered in sequential order 1-8 in order to distinguish between correct and incorrect receptacle choices in the chamber given a specific pattern. Correct responses in the chamber result in an all of the lights extinguishing and reinforcement delivery. Reinforcement was a 0.025-ml droplet of water delivered to the correct receptacle. Incorrect responses result in a time out procedure with a forced choice response. When an incorrect receptacle was selected, all of the lights in the chamber were extinguished except for the light in the correct receptacle. Rats could only select the illuminated receptacle, which was the correct receptacle. Following the nose-poke to the correct receptacle, reinforcement was delivered and the sequence then continued as if a correct response had been produced on the trial.

Training to Criterion

Rats ran 21-element patterns at a rate of 10 patterns per day for a total of 210 element responses per day during training. Rats were trained to a criterion of 90% or higher correct responses on all pattern elements for 10 patterns per day for 3 consecutive days. This is the same

criterion used by Chenoweth & Fountain (2015) and Fountain, Rowan, & Wollan (2013). After each rat reached criterion, the rat was randomly assigned to either the rule consistent pattern group or the violation pattern group. The day after receiving a saline injection 30 minutes before testing and demonstrating an ability to maintain a criterion of greater than 90% correct responses on all elements of the training pattern, rats were given 0.6 mg/kg of scopolamine 30 minutes before testing. At the time of test, rats were given the training pattern plus a novel 3-element chunk at the end of the pattern. This created a new 24-element serial pattern that was either rule consistent (chunk "812") or violated pattern structure (chunk "818"). Rats ran 10 patterns or were taken out of the apparatus an hour after being placed in the chamber. Males and females took an average of 61 and 62 days, respectively, to reach criterion in the training phase.

Statistical Analyses

ANOVAs were conducted using the SPSS statistical package (version 22.0, Chicago, IL). Results were significant if $p<.05$. For response analyses, data were analyzed by assessing the receptacles rats chose on each element of the pattern. Not all rats completed all 10 patterns on the day of the scopolamine challenge. Rats in the Rule Consistent group completed a mean of 8 patterns (males: 8.8, females 7.2) compared to the Violation group which completed a mean of 9.08 patterns (males: 8.83, females: 9.33). Because not all rats completed 10 patterns on the day of scopolamine administration, all scores are presented as means calculated as correct responses made out of the total number of responses for the completed number of patterns. Data were analyzed if a rat completed at least 4 patterns.

Results

Adding a Novel Chunk with Scopolamine Administration Increased Errors throughout the Pattern

We conducted a 2 x 2 x 8 x 3 (Sex x Pattern x Chunk x Element) repeated measures ANOVA to assess differences in pattern performance on the day when rats were given 0.6 mg/kg scopolamine and the novel 3-element chunk was added to the training pattern. The between-subject factors were Sex (male or female) and Pattern group. Pattern compared rats transferred to the rule-consistent chunk ("812") versus the violation chunk ("818"). Within-subject factors included Chunk (the eight 3-element chunks in the pattern) and Element (the three elements in each chunk). The ANOVA revealed significant main effects for Chunk ($F(7,126)=3.89$, $p=.001$, η^2 partial = .18), Element ($F(2,36)=97.56$, $p<.001$, η^2 partial = .84) and significant interactions for Sex x Element, ($F(2,36)=5.32$, $p=.01$, η^2 partial=.23), Pattern x Chunk ($F(7,126)=2.13$, $p<.05$, η^2 partial = .11), Pattern x Element ($F(2,36)=10.28$, $p<.001$, η^2 partial = .36), Chunk x Element ($F(14,252)=3.45$, $p<.001$, η^2 partial = .16), and Pattern x Chunk x Element ($F(14,252)=3.16$, $p<.001$, η^2 partial = .15). Other main effects and interactions were not significant ($p>.05$).

Planned comparisons for the significant Pattern x Element interaction depicted in Figure 2 shows that the Rule-Consistent group made significantly more errors (92.5% errors, SD=11.37) compared to the Violation group (61.39% errors, SD=25.56). There were significant differences between specific elements in the pattern which will be described by chunk number (ex: C1, C2,

C3, C4, C5, C6, C7, or C8) followed by the element number within each chunk (E1-3). For C1E3, the Violation group had a higher mean of 56.74% (SD=27.75) compared to the Rule-Consistent group which had a mean of 34.98 (SD=12.48), $p<.05$. C8E2 was also significant. Rats in the Rule-Consistent group had a higher mean error rate of 49.75% (SD=19.13) compared to the Violation group, which had a mean error rate of 31.46% (SD=20.35, $p<.05$). Finally, for the terminal element (C8C3), rats in the Rule-Consistent group made significantly fewer errors on the terminal rule-consistent element "2" (M=42.91%, SD=12.08) than the Violation group made on the terminal violation element "8" (M=90.00%, SD=10.44, $p<.001$).

Rats Made More Errors on the Violation Terminal Element than the Rule-Consistent Terminal Element of the Added Novel Chunk

Figure 3 displays male and female means for the added novel chunk. The terminal element of the added novel chunk was the only structural difference in the 24-element pattern given to the Rule-Consistent group and the Violation group. The 2 x 2 x 8 x 3 (Sex x Pattern x Chunk x Element) ANOVA yielded a significant Pattern x Element interaction, which revealed significant differences between the Rule-Consistent group and the Violation group for the terminal element. Since the focus of this experiment was to analyze performance between a rule-consistent terminal element or a terminal element that violated pattern structure, we conducted an ANOVA on male and female rat performance for the terminal element only. To analyze differences in performance on the terminal element, a 2 x 2 (Sex x Pattern) ANOVA was conducted to determine 1) if rats in the rule-consistent group differed in performance on the terminal element in comparison to the violation group, and 2) if males and females differed in performance on their respective terminal element. A significant difference between Pattern groups was found on the terminal element, in that the Rule-Consistent group (M=42.91%,

SD=12.08) made significantly fewer errors on the terminal element than rats in the Violation group (M=90.00%, SD=10.44), $F(1,18)=102.82$, $p<.001$, η^2 partial=.85. There were no significant sex differences and no significant Sex x Element interaction ($p>.05$).

Rats in All Groups Made Rule-Consistent Responses on the Terminal Element of the Added Novel Chunk

Past research supports that rats should not be able to learn a violation element introduced to a structured serial pattern in one day (Fountain & Rowan, 2000; Muller & Fountain, 2014), especially after being administered scopolamine (Fountain & Chenoweth, 2015). Administration of muscarinic anticholinergic drugs, such as scopolamine and atropine, can severely impair the performance of a learned violation element (Chenoweth & Fountain, 2015; Fountain, Rowan, & Wollan, 2013). Rats in the Rule-Consistent group made significantly fewer errors on the terminal element of the added novel chunk compared to the Violation group. We wanted to determine if rats in the Violation group were also making rule-consistent responses or if rats in this group were mostly making random errors since the violation element cannot be learned in one day. In order to determine if rats in the Violation group made rule-consistent responses in the novel added chunk, we assessed the responses rats made on the violation terminal element in comparison to responses rats made on the rule-consistent terminal element. For the terminal element, we analyzed the frequency of responses made at each receptacle in the chamber. This allowed us to see where rats were responding in the chamber on correct and incorrect responses. Figure 6 shows the mean percent frequency of responses for the terminal element as shown by sex and by pattern group for all eight receptacles on the scopolamine transfer day.

To analyze significant differences in receptacle responses for the terminal element, a 2 x 2 x 8 (Sex x Pattern x Receptacle) ANOVA was conducted. For the analysis, we calculated the number of responses made on each receptacle for both correct and incorrect responses on the terminal element. As shown in Figure 4, results of the ANOVA revealed a significant within-subjects main effect for receptacle number ($F(7,126)=54.75$, $p<.001$, η^2 partial=.75), meaning that differences were present for the amount of responses on different receptacle numbers. Specifically, receptacle 2 had higher responses across both pattern groups and both sexes in comparison to all other receptacles for responses made on the terminal element ($p<.001$). This means that both male and female rats in the violation and rule-consistent groups were making mostly rule-consistent responses on the terminal elements of their novel chunks.

In addition to the amount of receptacle 2 responses being significantly different from all other receptacle response rates, receptacle 6 was significantly different than receptacle 1 ($p<.01$); receptacle 7 was significantly different than receptacle 8 ($p<.05$) and receptacle 1 ($p<.01$); receptacle 8 was significantly different than receptacle 4 ($p<.05$); receptacle 1 was significantly different than receptacle 4 ($p<.05$), and receptacle 5 ($p<.05$). Sex x Receptacle within-subjects interaction was also significant ($F(7,126=2.11$, $p<.05$, η^2 partial=.11). No other main effects or interactions were significant ($p>.05$).

Comparison Between the Last Chunk of Training Pattern and the Novel Chunk

Figure 3 shows rat performance across all chunks of the pattern. In order to determine if rats learned the novel chunks independently or made similar responses as a previously learned chunk, we compared performance on the last chunk of the training pattern (chunk "781") to the added novel chunk on the day of scopolamine administration. We conducted a 2 x 2 x 2 x 6 (Sex x Pattern x Chunk x Element) ANOVA to answer this question. Chunk was a within-subject

factor that compared a rat's performance on the last chunk of the training pattern (chunk "781") to the added novel chunk ("812" or "818"). No significant between-subject differences were observed. The lack of overall between-subject differences across the two chunks demonstrates that rats treated both chunks similarly. This means that rats did not treat the added novel chunk like it was novel despite the violation group's terminal element violating pattern structure.

Other results of the ANOVA revealed a main effect of Chunk ($F(1,18)=36.78$, $p<.001$, η^2 partial=.67). Chunk "781" had a significantly lower mean error rate (52.31%, SE=3.33) than the mean error rate for the novel chunk (64.10%, SE=2.72). In addition to chunk, there was also a main effect of element $F(2,36)=25.33$, $p<.001$, η^2 partial=.59). The chunk-boundary element in each chunk (first element of each chunk) had significantly more errors (M=79.88, SE=5.39) than both of the within-chunk elements (element 2 in each chunk - M=40.93, SE=4.44, $p<.001$; element 3 in each chunk M=53.80, SE=2.68, $p<.01$). There was a significant interaction for Pattern X Chunk ($F(1,18)=17.16$, $p<.001$, η^2 partial=.49), Pattern X Element ($F(2,36)=4.99$, $p<.05$, η^2 partial=.22), Chunk X Element ($F(2,26)=8.30$, $p<.001$, η^2 partial=.32), and Pattern X Chunk X Element ($F(2,36)=11.68$, $p<.001$, η^2 partial=.39). Sex X Element, Sex X Pattern X Element, Sex X Pattern X Chunk, Sex X Chunk X Element, and Sex X Pattern X Chunk X Element were not significant ($p>.05$).

Figure 5 shows rats' receptacle responses on the last element of the training pattern (chunk "781", element "1") on the day of scopolamine administration. Receptacle responses on the last element of the training pattern and on the terminal element were compared to assess if rats were making rule consistent responses across chunks. If rats were making a similar pattern of responses at the receptacles for these two elements, then it would be evident that a similar strategy was being employed. This would be evidence that rats were using the same rule to make

consistent responses across the two chunks. A 2 x 2 x 2 x 8 (Sex x Pattern x Element x Receptacle) was conducted to assess Element as a within-subjects factor to compare a rat's response on the last element of the training pattern ("1") compared to the same rat's response on the terminal element of the added novel chunk (mostly "2" across both the violation group and the rule-consistent group). For this analysis, if rats' level of responding does not differ between the two elements, then the mean rate of responding at receptacle "1" should not differ from receptacle "2", since "2" was the most frequently chosen receptacle for rats in both groups for the terminal element of the added novel chunk.

The ANOVA revealed a significant main effect for receptacle ($F(7,126)=57.23$, $p<.001$, η^2 partial=.76). Figure 5 displays the frequency of responses rats made at receptacles. Bonferroni pairwise comparisons revealed that when collapsed across chunks, receptacle 1 responses were significantly different than all other receptacles ($p<.001$), except for receptacle 2. Receptacle 2 responses were significantly different than all other receptacles expect for receptacle 1 ($p<.001$; except for receptacle 8, $p<.01$). As shown in Figure 5, receptacle 8 responses were significantly different than all other receptacle responses (receptacles 1,3, & 4, $p<.001$; receptacles 2,5, & 6 $p<.01$), except for receptacle 7. In addition, receptacle 5 and receptacle 7 were significantly different, $p<.01$. In addition to the main effect of receptacle, a significant interaction for Element X Receptacle ($F(7,126)=46.83$, $p<.001$, η^2 partial=.72) reveals that rats were making significantly more responses at receptacle "1" when making the last element response of the training pattern (M=57.47, SD=4.47) and then more receptacle "2" responses when making the terminal element response (M=53.65, SD=3.73). No other main effects or interactions were significant ($p>.05$).

We plotted the first pattern that rats ran on the day of scopolamine administration focusing on responses made only on the last element of the added chunk. We did this to ensure that results of our analyses assessing responses across all patterns were not biased towards rats' responses made after several patterns worth of experience with the added chunk. By looking at the first pattern responses on the last element of the added chunk we are able to match the trend of responses between rats' first experience with the terminal element of the novel chunk to overall performance on the terminal element of the novel chunk. Figures 6a and 6b display rat responses on the terminal element of the training pattern for the first pattern on scopolamine day. Rats first pattern responses for the terminal element of the training pattern (element 1) show that rats made the most responses at receptacle 1 on the first pattern. This demonstrates that despite scopolamine administration causing higher errors, rats were able to make the trained rule-consistent response. Figures 7a and 7b display rats' responses on the terminal element of the added novel chunk for the first pattern on scopolamine day. Similar to the results found for rats' first experience with the last element of the training pattern, rats made mostly rule-consistent responses at their first experience with the last element of the novel added chunk.

Sex Differences in Chunk-Boundary and Receptacle Choices Relative to Chamber Location

Sex differences were observed in chunk-boundary and receptacle choices relative to chamber location. Though these effects are of interest, they are not germane to the question that is the focus of this paper, namely, rats' ability to extrapolate rules while under muscarinic cholinergic suppression, so the results are described separately in Appendix 1.

Discussion

The goal of this study was to find evidence for rule extrapolation under the conditions of muscarinic cholinergic suppression. Overall, muscarinic cholinergic suppression via scopolamine did not cause an impairment in rule extrapolation. Both male and female rats were able to extrapolate the abstract rule from the training pattern to make rule-consistent responses on the terminal element of the novel chunk. This finding was not limited to the Rule-Consistent group in that rats in the Violation group also made mostly rule-consistent responses on the violation element despite never being reinforced for doing so. In addition to this evidence of rule extrapolation, we also found that the number of rule-based responses on the terminal element of the novel added chunk was similar to the number of correct rule-based responses on the terminal element of the training pattern. That is, rats made as many rule-based (extrapolation) responses on the violation element of the novel added chunk (Chunk 8, Element 3) as they did on the already-trained corresponding element of the chunk just before the novel added chunk (namely, Chunk 7, Element 3). The results extend those of Fountain & Hulse (1981) and Fountain & Rowan (2000) that showed that rats can extrapolate a learned rule to novel elements, in this case even under scopolamine-induced muscarinic cholinergic suppression.

Earlier research with the SMC task including training to criterion followed by a drug challenge, the addition of a novel chunk, or both concurrently (Chenoweth & Fountain, 2015, 2016; Fountain & Rowan, 2000; Fountain, Rowan, & Wollan, 2013) has shown that these

manipulations produce widespread effects in serial patterns. To better understand the cause of an increase in both within-chunk and chunk-boundary errors throughout the pattern in the current study, we must consider that this experimental design consists of two major procedure changes that rats experienced on testing day. The effects of adding a new 3-element chunk, regardless of whether or not it was rule-consistent, may have had additive or even multiplicative effects when combined with scopolamine exposure. Rat stimulus-response or discrimination learning mechanisms employed to make chunk-boundary responses may be more highly impacted by structural changes to a learned pattern than abstract rule learning mechanisms employed to make within-chunk responses, but both are sensitive to the addition of elements despite the structure of the elements added (Fountain & Rowan, 2000; Fountain, Rowan, & Wollan, 2013). For example, in the Fountain & Rowan (2000) MK-801 experiment, which contained the same procedure as the current experiment, when rats were given MK-801 on a day when rats also received a novel chunk, chunk-boundary errors did increase regardless of whether or not the added novel chunk was rule-consistent or contained an element that violated pattern structure. Within-chunk errors only increased for rats that received MK-801 and a novel added chunk that contained an element that violated pattern structure (Fountain & Rowan, 2000). In contrast to rats exposed to MK-801, rats that received saline did not have errors for chunk-boundary elements or within-chunk elements that differed from criterion day when either novel chunk was added. Instead, the researchers observed that these elements were unaffected despite the addition of an added novel chunk (Fountain & Rowan, 2000). The results of both manipulations applied concurrently in the current experiment most resemble the results observed under the conditions in Fountain & Rowan (2000) where rats received both the NMDA receptor blocking drug, MK-801, and an added violation chunk as used in the current study. In both cases, rats showed strong

extrapolation of the pattern in the novel added chunk despite suppression of the target neurotransmitter systems. Thus it follows that extrapolation in this specific SMC test does not depend on normal NMDA receptor or muscarinic receptor function.

It is also not known how the addition of a novel chunk might change the utilization of a previously used learning mechanism and how this might interact with muscarinic cholinergic neurotransmission. Neither the MK-801 experiment conducted by Fountain & Rowan (2000) nor the current experiment were designed to identify and characterize potential deficits that might be caused by drug administration, by adding elements to the training pattern, or by a combination of the two. The deficits seen in both the current experiment and the Fountain & Rowan (2000) experiment must be due to memory issues caused by a combination of pattern structure change and the administration of drugs known to contribute to memory impairments. The addition of elements to the training pattern can cause an increase in rat memory load whereas the administration of MK-801 and scopolamine block memory of what was learned. This is one potential explanation for why we see higher errors when both the added chunk and drugs are administered on the same day. A future experiment will need to explore how muscarinic cholinergic suppression might interact with the addition of elements or chunks of elements. If the rules used to make responses in the added chunk are not necessarily disrupted, it is perhaps the addition of more elements that causes an increase in memory load which translates into higher errors throughout the pattern.

This is the first study to examine extrapolation in both sexes with results indicating that male and female rats did not differ in terms of the effects of muscarinic cholinergic suppression on pattern extrapolation. Previous studies using this paradigm with muscarinic cholinergic suppression have used only males (Chenoweth & Fountain, 2015; Fountain, Rowan, & Wollan,

2013). However, Pickens and colleagues (2013) used this paradigm to assess the effects of adolescent nicotine exposure, that is, exposure to a nicotinic acetylcholine agonist, on adult rat learning in both male and female rats. Sex differences in acquisition were observed in control groups: saline-injected females were learned both chunk-boundary elements and a violation element slower than saline-injected males. In the current study, some significant sex differences were found (see Appendix 1). Specifically, females in the rule-consistent group made significantly more errors on the first element of the pattern than males. A follow-up of this finding was conducted in the form of a response analysis to assess if females were picking a different receptacle to the extent that an underlying mechanism could be determined. However, the errors made on the first element were spread across receptacles yielding no information on what caused this error inflation for females on the first element of the pattern. Despite this sex difference, there was not a significant sex difference on rat performance for the terminal elements in the added novel chunks. This finding yields new information, in that both male and female rats are capable of applying a previously learned rule to novel information.

One future direction for this line of research should work to utilize patterns with different rules to assess if serial pattern rule learning will always transfer to novel information or if it is limited to the specific pattern used in this study. In the current experiment, the pattern contained chunks organized in "runs" of elements, meaning that within each chunk rats are making a continuous run of responses (e.g. 123-234-345…). Previous research using this paradigm has assessed acquisition of "trill" chunks (e.g. 121-232-323...) in comparison to runs chunks (Fountain & Rowan, 1995a). In an experiment conducted by Fountain & Rowan (1995a), rats were trained on either a 24-element pattern composed of chunks made of runs or chunks made of trills. Both a pattern composed of runs and a pattern composed of trills are perfectly structured,

meaning that a predicable structure is present throughout the pattern. In comparison to the two perfect patterns, researchers assessed learning of a runs pattern with violation (123-234-345-456-567-687-781-818) and a trills pattern with violation (121-232-3343-454-565-676-787-812). The results showed that rats could learn both a perfect runs pattern and a perfect trills pattern, however, rats in the trills pattern group made significantly more errors across all elements compared to rats in the runs pattern group (Fountain & Rowan, 1995a). Rats that received the runs pattern with a violation element or the trills pattern with a violation element made significantly more errors on the violation elements compared to other elements in the patterns (Fountain & Rowan, 1995a). Because a trills pattern is harder to learn than a runs pattern due to the complexity of the rule learned, it would be interesting to see if rats trained on a trills pattern that are transferred to a novel chunk would behave similarly to rats on a runs pattern. Since rats were able to make a rule-consistent response on an added novel runs chunk, we expect that this effect holds when rats are given a more challenging rule. We would predict, based on data from the Fountain & Rowan (1995a) experiment, that rats would find the application of a learned trills chunk rule more difficult than a runs chunk rule. Other future directions in this line of research will seek to establish how much flexibility is in the pattern rules learned under the condition of an anticholinergic drug. For example, how flexible are rules learned about pattern structure when the structural rule is changed? To answer this question, we will train rats first on a perfect runs pattern, for example, while administering daily injections of scopolamine prior to training, then transfer to a trills pattern, and other groups would receive the opposite order. Such experiments will allow us to better understand how muscarinic cholinergic suppression during training impacts cognitive flexibility for elements that reflect either discrimination learning or abstract rule learning.

Because other studies have demonstrated that muscarinic cholinergic suppression can impair the encoding of a rule (Chenoweth & Fountain, 2015) and serial pattern rule learning does not seem sensitive to muscarinic cholinergic suppression, it is still unclear how the type of rule learning we study in serial pattern learning differs from rule learning in other tasks. The current study has ruled out that muscarinic cholinergic systems play a role in serial pattern rule extrapolation, however, we are still unsure as to what neurotransmitter systems do play a role in serial pattern rule extrapolation or which brain systems might be involved in rule learning and rule extrapolation processes. All previous research in the SMC task has assessed the central effects of systemic injections of drugs that can pass through the blood-brain barrier and thus affect neurotransmission throughout the brain. Future research must begin to target areas of the brain known to be important to sequential learning, such as the hippocampus and related structures, the basal ganglia, and the frontal cortices (Blokland, Honig, & Wijnand, 1992; Chaveau et al., 2009; Fast et al., 2016; Truman, Brooks, & Dunnett, 2005; Winters et al., 2010). In order to target appropriate brain regions, it is imperative to develop a better understanding of the role of more than just muscarinic cholinergic neurotransmission in specific serial pattern learning mechanisms.

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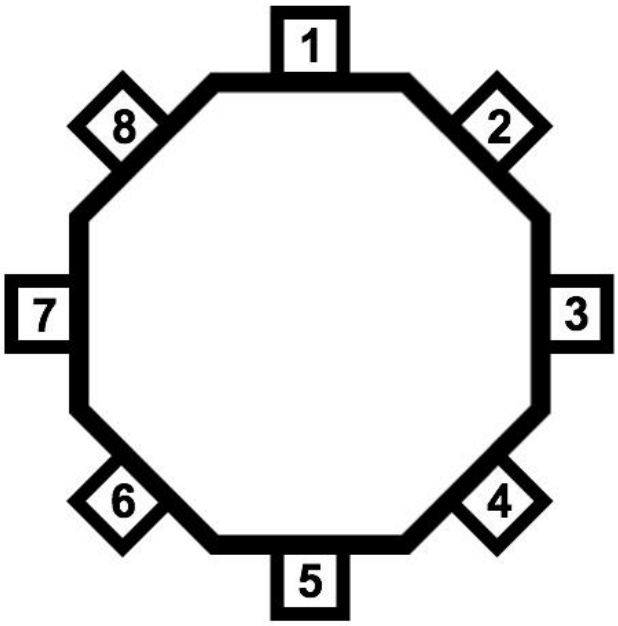

Figure 1. Octagonal chamber layout used in the Serial Multiple Choice (SMC) task. Rats learn to nosepoke in 8 receptacles in a sequential pattern.

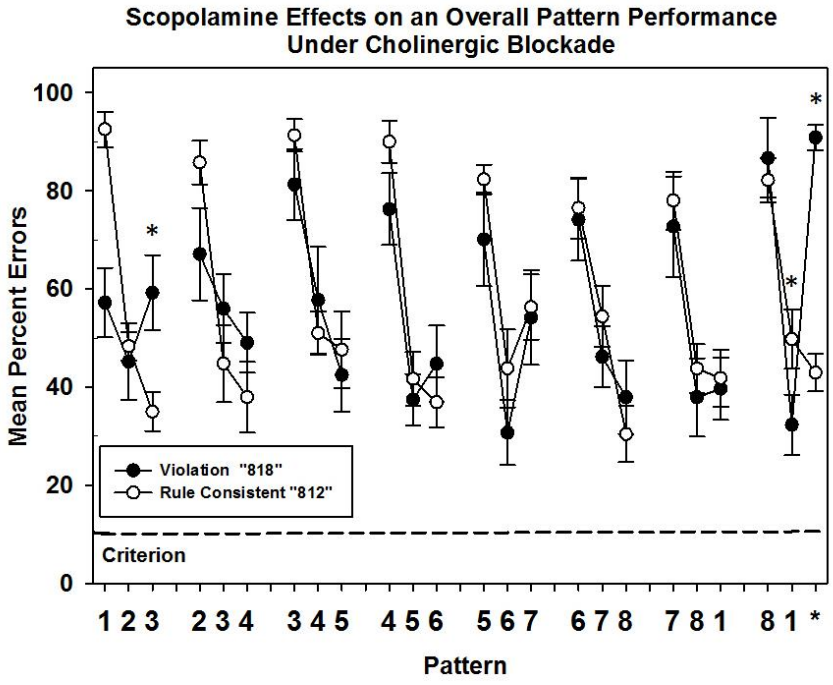

Figure 2. Mean percent errors collapsed across sex as shown by pattern on the day of transfer when rats were given a new chunk of information that was either rule-consistent (chunk "812") or violated pattern structure (chunk "818"). The X-axis is the training pattern in addition to the added chunk "8-1-*". The Y-axis is the mean percent correct. Groups are displayed by pattern group. A line at 10% errors shows the highest level of errors on criterion day. *p<.05.

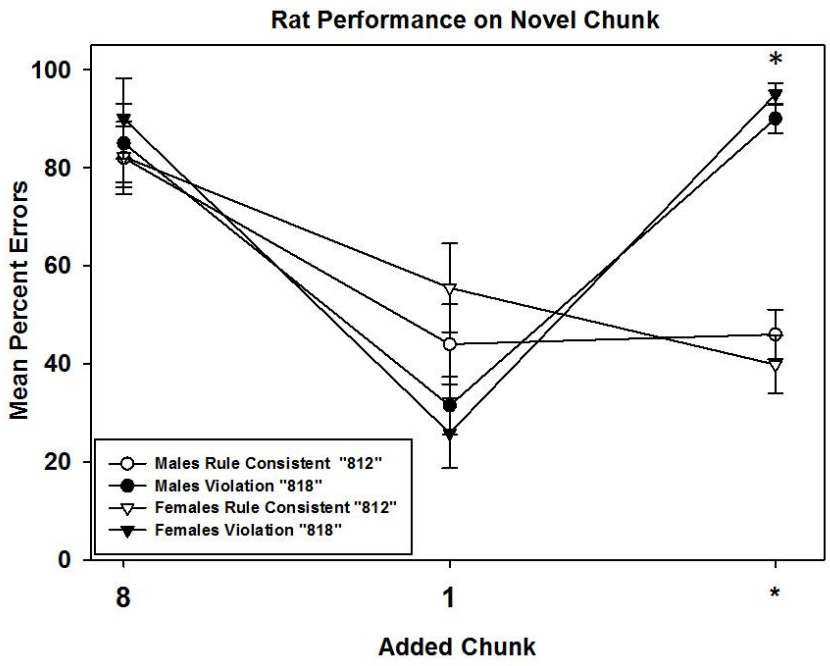

Figure 3. Mean percent errors for males and females on the chunk added to the training pattern on the day of the scopolamine challenge. Rats received 0.6 mg/kg of scopolamine on the day the chunk was added. Rats in the Rule-Consistent groups made significantly fewer errors on the terminal element compared to rats in the control group, $p<.001$. No Sex or Sex X Group differences were observed ($p > .05$).

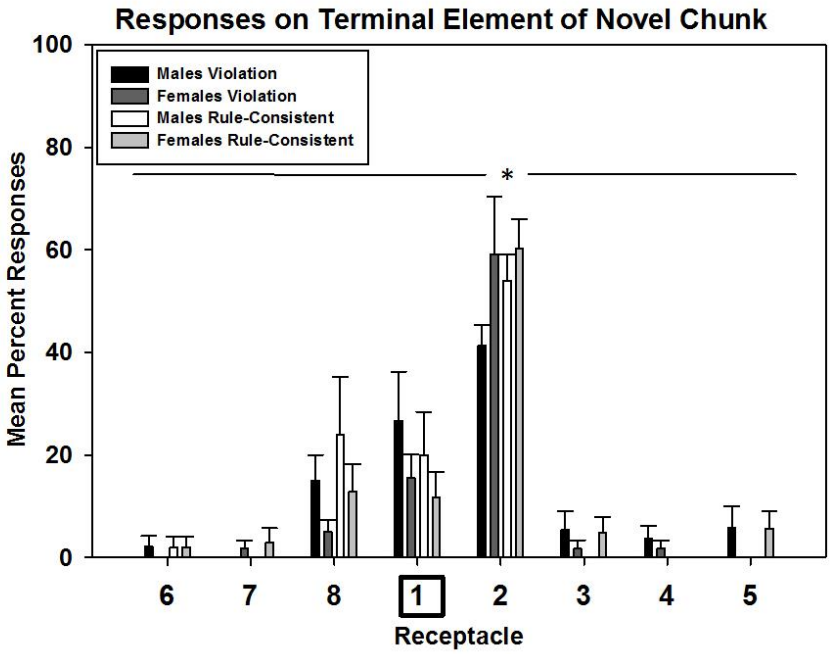

Figure 4. Frequency of responses for the last element of the added chunk. For both groups, rats are at the 1 receptacle position prior to making the last element response (shown in the square). Overall, rats in both groups make mostly responses at the 2 receptacle, showing that under scopolamine rats persist to apply a previously learned rule regarding pattern structure. Males (n=5, 6), Females (n=5, 6).

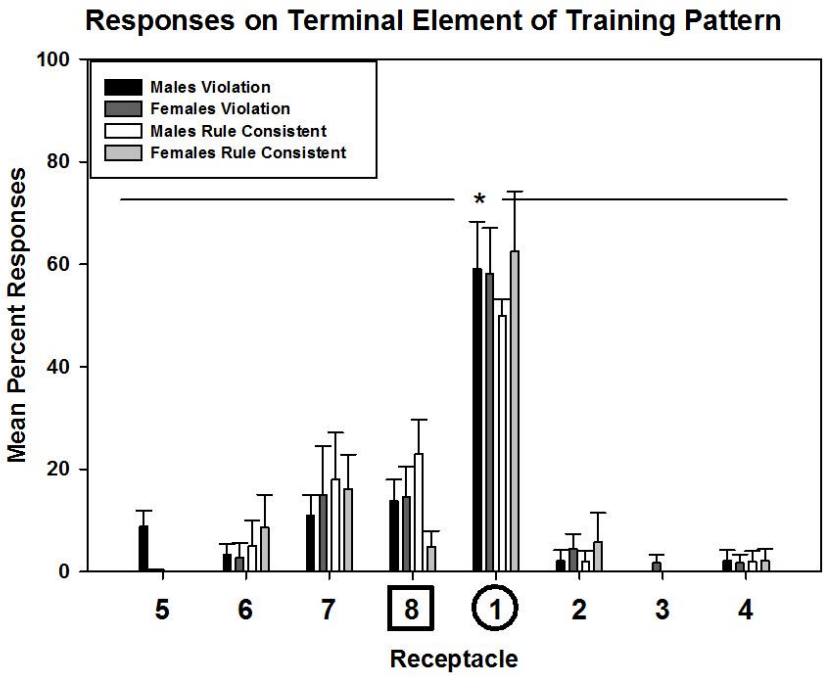

Figure 5. Rat responses on the last element of the training pattern on scopolamine day. The square marks receptacle 8, which is the receptacle rats are at prior to making the response at the 1 receptacle. The circle around receptacle one shows that this is the target receptacle for correct response.

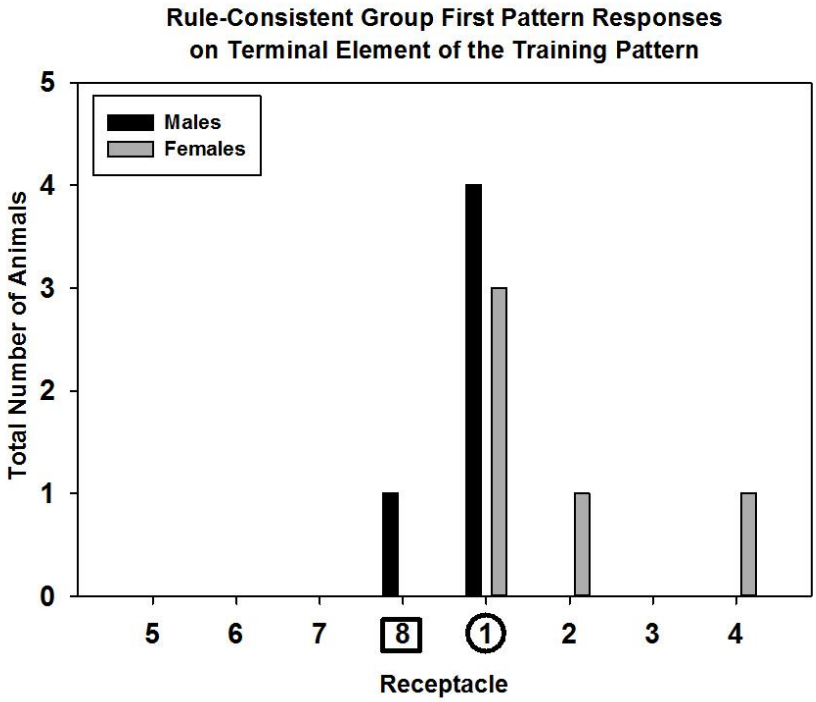

Figure 6a: First responses rats made on the terminal element of the training pattern on scopolamine day. Male and female rats were at receptacle 8 (shown in the square) and the correct element response was to choose receptacle 1 (shown in the circle). The Y-axis reflect the number of animals per group. Rats in the Rule-Consistent group (n=5 per sex).

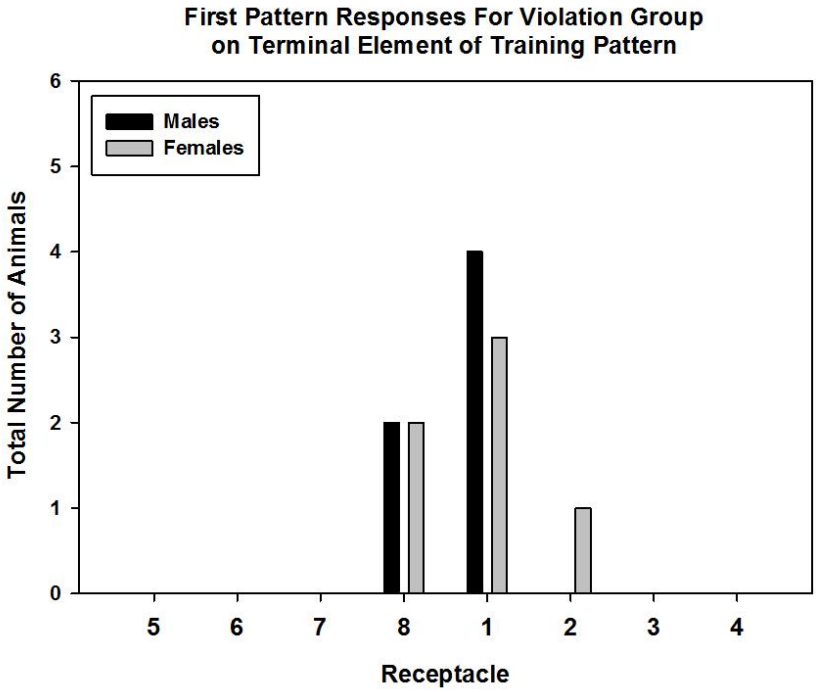

Figure 6b: First responses rats made on the terminal element of the training pattern on scopolamine day. Male and female rats were at receptacle 8 (shown in the square) and the correct element response was to choose receptacle 1 (shown in the circle). The Y-axis reflect the number of animals per group. Rats in the Violation group (n=6 per sex).

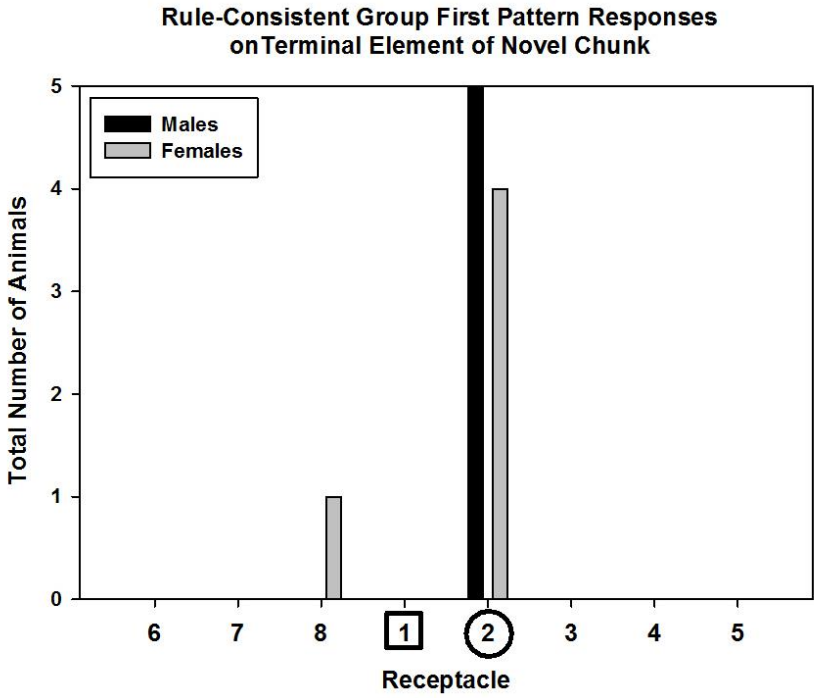

Figure 7a: First responses the Rule-Consistent group male and female rats made on the terminal element. Y-axis reflect the number of animals per group. Rats were at receptacle 1 (shown in the square) and the terminal element response was 2 (shown in the circle) which was rule-consistent with the rest of the pattern.

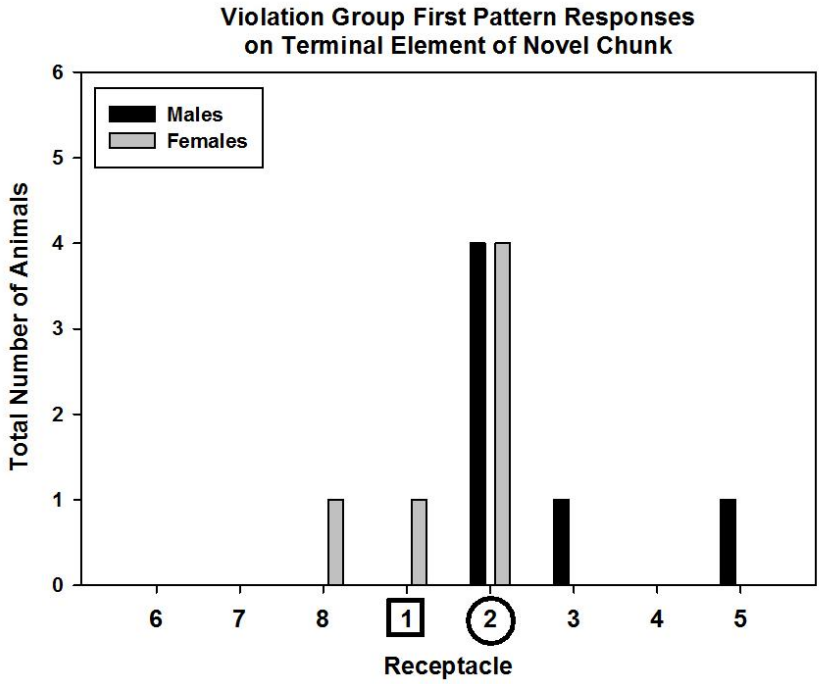

Figure 7b: First responses male and female rats in the Violation group made on the terminal element. Y-axis reflect the number of animals per group. Rats were at receptacle 1 (shown in the square) and the terminal element was 8 (shown in the circle), which violated the structure of the pattern.

Appendix:

Sex Differences in Chunk-Boundaries and Receptacle Choices Relative to Chamber Location

The repeated measures ANOVA did not find a significant between-group difference for Sex for overall pattern performance. However, planned comparisons for the significant Sex x Element interaction indicated that females had a higher mean error rate on chunk-boundary elements (first element of each chunk) (M=83.62%, SD=5.53) compared to males (M=75.80%, SD=5.53). See Figure 8 for male performance and female performance split by sex and group.

Across all elements of the pattern, males in the Rule-Consistent group had a mean error rate of 59.67% (SD=6.15), while males in the Violation group had a mean of error rate of 57.48% (SD=5.61). Females in the Rule-Consistent group had a mean error rate of 55.73% (SD=6.15), and in the Violation group a mean error rate of 54.76% (SD=5.61) across all elements of the pattern. In addition to females having higher errors on chunk-boundary elements, females performed significantly worse on the first element of the pattern. Bonferroni pairwise comparisons revealed that females (M=85.34% errors, SE=5.06) made significantly more errors that males (M=64.38%, SE=5.06) on the first element of the pattern (see Figure 11). There was also a significant main effect for Pattern ($F(1,21=24.26$, $p<.001$, η^2 partial = .57).

In order to further analyze the significant differences between sexes and pattern groups on the first element of the pattern, a 2 x 2 x 8 (Sex x Pattern x Receptacle) ANOVA was conducted on the location of responses made on the first element of the pattern. Sex and Pattern

were between-subject factors. The factor Receptacle was a within-subject factor defined as the 8 receptacle locations in the chamber at which rats could respond during each trial. As shown in Figure 9, the analysis found no significant between-groups differences for Sex or Pattern. A significant main effect of Receptacle was found ($F(7,126)=5.63$, $p<.001$, η^2 partial $=.24$). Bonferroni pairwise comparisons revealed that receptacle 1 (M=28.68, SE=3.93) responses were significantly different than receptacles 3 (M=11.18, SE=1.96), 4 (M=6.65, SE=2.37), 6 (M=8.46, SE=2.43), 7 (M=7.72, SE=2.41), and 8 (M=10.58, SE=2.45) (*ps*<.05). This means that male and female rats responded most at receptacle 1 (the correct response) on the first element of the pattern, compared to receptacles 3, 4, 6, 7, and 8. There were no other significant main effects or interactions ($p>.05$). Figure 11 displays the frequency of responses at each receptacle location rats made for the first element of the pattern. Responses seem to be fairly random and this is supported by the lack of significant findings for between-subject factor differences in the ANOVA.

The overall pattern ANOVA revealed that rats made significantly more errors on the first element of each chunk which is the chunk-boundary element. Bonferroni paired comparisons revealed that the first element of each chunk (chunk-boundary elements) had a significantly higher mean error rate of 79.71% (SD=3.91) in comparison to the within-chunk elements (element 2 M = 44.69%, SD = 3.30, $p<.001$; element 3 M = 45.69%, SD=2.71, $p<.001$). As displayed best in Figure 2, chunk-boundary errors on the day of scopolamine had an overall mean of 79.71% errors (SE=3.91) which is comparable to the amount of errors made on the violation element for the violation group (M=90.00%, SD=10.44). This suggests that rats were treating a highly trained structured element similarly to a novel rule-violating element once scopolamine was administered.

To fully evaluate the types of responses rats made on chunk-boundary responses, we conducted a 2 x 2 x 8 x 8 (Sex x Pattern x Chunk-Boundary x Response) repeated measures ANOVA. In order to compare receptacle responses across different chunk-boundaries, we categorized 8 responses that could be made on the chunk-boundary element relative to the correct response prior to making the chunk-boundary response (last within-chunk element in a chunk). We chose this location because even if rats did not make the correct response at element 3 within a chunk, the correction procedure would force rats to end up in this location before they could make a chunk-boundary response. Using this within-chunk response as a starting point, we assessed the percentage of responses made at all receptacles across all chunk-boundary elements. For this analysis, Chunk-Boundary was a within-subject factor that represented a rat's response across chunk-boundaries in the pattern (chunk-boundaries 1-8). Response was a within-subjects factor that assessed a receptacle location in the chamber the rat could choose. See Figure 12 for a pictorial representation of the receptacle layout of responses used for the analysis. In this pictorial representation, instead of numbering the receptacles 1 through 8, we now have labeled responses relative to the rat's location prior to making the chunk-boundary response. For example, in the pattern "123-234" rats were at the "3" receptacle location, as it is the last element of the first chunk, prior to making the chunk-boundary response at receptacle "2". On Figure 13, the asterisk at receptacle "P" represents the position of the rat on the last within-chunk element before a chunk-boundary (receptacle "3" in the previous example). We chose this location because even if a rat made an error, the rat would undergo the correction procedure and would be at this position prior to choosing the next receptacle. The "P" at this location is representative of a perseveration response. Perseverations mean that the rat made a response at the last correct location rather than moving forward or backward to another receptacle. In previous studies using

the SMC task to assess chunk-boundary learning, we found that half or more of the errors made on chunk-boundaries were perseverations at the last correct receptacle (Muller, 2010). This means that instead of rats' moving left one receptacle to make the chunk-boundary response, rats were staying at the last correct receptacle. The correct chunk-boundary response, labeled "C" was one to the left of the "P" receptacle. Moving forward from "P" are receptacles "+1", "+2", and "+3", which represent rats moving forward one, two, or three receptacles, respectively. Moving backwards from "P" are receptacles "-2", "-3", and "-4", which represent moving backwards two, three, or four receptacles, respectively. We cannot assume on receptacle responses "+2", "+3", "-2", "-3", or "-4" that rats moved a specific direction backwards or forwards. Instead, these are categorical names given to receptacles relative to "P" so that we can analyze across chunks the spatial array of receptacle choices rats were making. Rats that made responses at "+1" were actually making responses that would be rule-consistent with a within-chunk response since relative to the rat's current location it would be moving forward to the right one receptacle consistent with the abstract rule for within-chunk responses. For this analysis, we were most interested in determining if errors made were consistent with past research (namely, mostly perseverations) or if scopolamine was potentially causing rats to make more +1 rule-type responses.

For the 2 x 2 x 8 x 8 (Sex x Pattern x Chunk x Response) repeated measures ANOVA, the between-subject factors were Sex and Pattern group. Within-subject factors were Chunk-boundary elements (1-8) and Response (C, P, +1, +2, +3, -2, -3, -4). Results of the ANOVA indicated that there was a significant main effect for Response, $F(7,126)=18.15$, $p<.001$, η^2 partial=.50, in which Bonferroni pairwise comparisons reveal that rats made significantly more correct responses on chunk-boundary elements than -2 ($p<.05$), -3 ($p<.05$), or -4 ($p<.01$)

responses. This means that rats rate of correct responses at chunk-boundaries did not significantly differ from perseverations, +1 (within-chunk) responses, +2 responses, and +3 responses. A significant Chunk-Boundary X Response interaction was found, $F(49,882)=3.00$, $p<.001$, η^2 partial=.14. See table 1 for the means and standard errors for chunk-boundary responses made relative to the correct receptacle. No other main effects or interactions were significant, $p>.05$.

Chunk-Boundary Discussion

Serial pattern rule learning does not require muscarinic cholinergic neurotransmission but serial pattern discrimination learning might. The current study assessed discrimination learning in terms of chunk-boundary element stimulus-response learning. Unlike the experiment by McGaughy, Koene, Eichenbaum, & Hasselmo (2005), we did find that discrimination learning was impaired when scopolamine was administered prior to testing after discrimination training. Scopolamine caused errors on chunk-boundaries, to increase to errors at approximately 80% compared to errors being under 10% on the day before drug administration. For both novel chunk groups, the level of errors made on chunk-boundaries on the day of scopolamine administration was not significantly different than the level of errors rats made on the violation element. This finding was unexpected because previous studies demonstrated that performance on chunk-boundary elements learned to a high criterion level should be remain intact despite muscarinic cholinergic suppression (Chenoweth & Fountain, 2016; Fountain, Rowan, & Wollan, 2013). Therefore, it was not predicted that highly learned chunk-boundary elements would be impaired to the level of a novel element that violates pattern structure.

In a study conducted by Chenoweth & Fountain (2015), rats that were trained to a criterion level of less than 10% errors on a 24-element serial pattern with within-chunk, chunk-

boundary, and violation elements. The day following criterion, rats were given an i.p. injection of atropine (a muscarinic anticholinergic drug similar to scopolamine), which did cause an increase in errors for all elements. However, the level of errors was approximately 50% for chunk-boundary elements, which is substantially lower than the approximately 80% error rate found in this study. So while Chenoweth & Fountain (2015) found similar results as McGaughy, Koene, Eichenbaum, & Hasselmo (2005), in that after rats learned a discrimination scopolamine had little impact on task performance, the current study does not support these results.

It can be ruled out that the high amount of errors made on chunk-boundary elements was the result of scopolamine impairment of rats' sensitivity to pattern structure. If rats were not sensitive to pattern structure, we would see random errors throughout the pattern or good performance for within-chunk elements but not chunk-boundary elements. This would mean that rats could apply a "+1" rule of directional movement throughout the chamber but could possibly have impaired stimulus-response mechanisms to make chunk-boundary responses. In this case, we would get a similar pattern of errors seen in this experiment where rats are making very high errors on chunk-boundary elements to the extent that it appears that rats have forgotten how to make the chunk-boundary response. However, we can rule out this lack of pattern sensitivity by looking at a previous study by Chenoweth & Fountain (2016) which used probe patterns to test rat sensitivity to structure. Chenoweth & Fountain (2016) trained rats to the same criterion of this study (less than 10% errors) on the 24-element serial pattern that was used on the day of scopolamine administration (123-234-345-456-567-678-781-818). She then gave rats 50 mg/kg of atropine (a muscarinic antagonist) or saline and assessed rat performance on the 24-element training pattern with occasional probe patterns. The goal of one of the probe patterns was to assess if rats were still sensitive to chunk-boundary elements. This probe pattern gave a string of

ordered elements “12345678”. If rats were not sensitive to chunk-boundary structure and were utilizing an easy directional rule to continue forward and make within-chunk responses, then errors should be just as low at element 4 (where the second chunk should begin) as element 3 (last within-chunk element in the first chunk). Chenoweth & Fountain (2016) found that rats were sensitive to chunk-boundary pattern structure, that is, both atropine groups and saline groups had a similar increase of errors at element 4 in the probe pattern.

Another probe used in the Chenoweth & Fountain (2016) experiment removed the 3-second phrasing cues from the pattern after training rats to less than 10% errors with a phrasing cue. Cue removal significantly increased errors made on chunk-boundary elements for both rats administered atropine or administered saline and these errors persisted throughout the pattern. Overall, muscarinic cholinergic suppression did not result in a decrease in sensitivity to chunk-boundary placement in the pattern nor did it affect rats’ ability to attenuate to the phrasing cue (Chenoweth & Fountain, 2016). The probe removing chunk-boundaries by increasing the run of elements (e.g. 12345…) demonstrated that despite being administered scopolamine rats were sensitive to where a chunk-boundary should occur in the pattern. The probe that removed the phrasing cues demonstrates that despite being administered scopolamine, rats anticipated the discriminative pause in the chamber to signal when to make a chunk-boundary response. Taken together with the first probe pattern, this means scopolamine does not impair rats’ ability to know when and where in a serial pattern to make a chunk-boundary response. This is evidence that scopolamine administration cannot cause the high chunk-boundary errors seen in the current study. Instead, it is most likely that scopolamine and the addition of elements to the training pattern had additive effects on rats’ ability to perform chunk-boundaries.

The addition of adding a novel chunk to the end of the training pattern could cause high error rates on the first element of the pattern, however, an analysis of the receptacles rats made responses at on the first element of the pattern did not yield any insight. Fountain & Rowan (2000) also found that when a novel chunk was added to a training pattern that errors increased on the first element of the pattern. In the current study, the lack of significant differences found between the amount of responses at each receptacle seems to suggest that rats were making random errors and not strategy-based errors. Females in the Rule-Consistent group had more errors than any other group, but no rationale could be determined from the data since the placement of errors throughout the chamber on the first element of the pattern was not significantly different between groups. Based on the pattern structure, rats in the Rule-Consistent group were spatially set-up to more easily make a correct response at the first element. By ending with the chunk "812", the next chunk response of "123" follows all other rules learned and applied at other points in the pattern. This experiment was not designed to assess the strategy behind rat responses for the first element of the pattern, therefore, it is currently hard to discern why no female rats in the Rule-Consistent group could make a correct response on the first element of the pattern. Rats both started and ended the training pattern at receptacle 1. Because rats started and ended the training pattern at receptacle 1, it should seem likely that all rats would perseverate towards the end and beginning of the pattern at the 1 receptacle. However, Figure 9 shows that this was not what was observed.

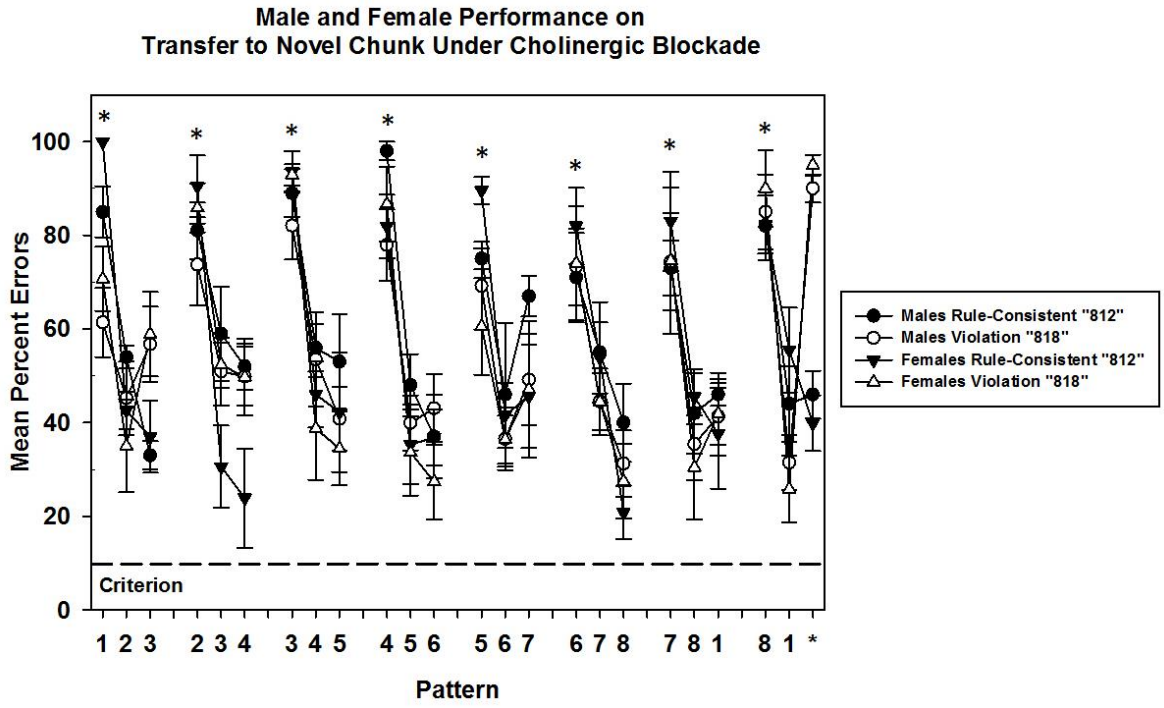

Figure 8. Male and female performance on the day of the scopolamine challenge when a new 3-chunk element was added. For the Rule Consistent group, n=10; 5 males and 5 females. For the Violation group, n=12; 6 males and 6 females. Significance stars represent the significant Sex X Element interaction.

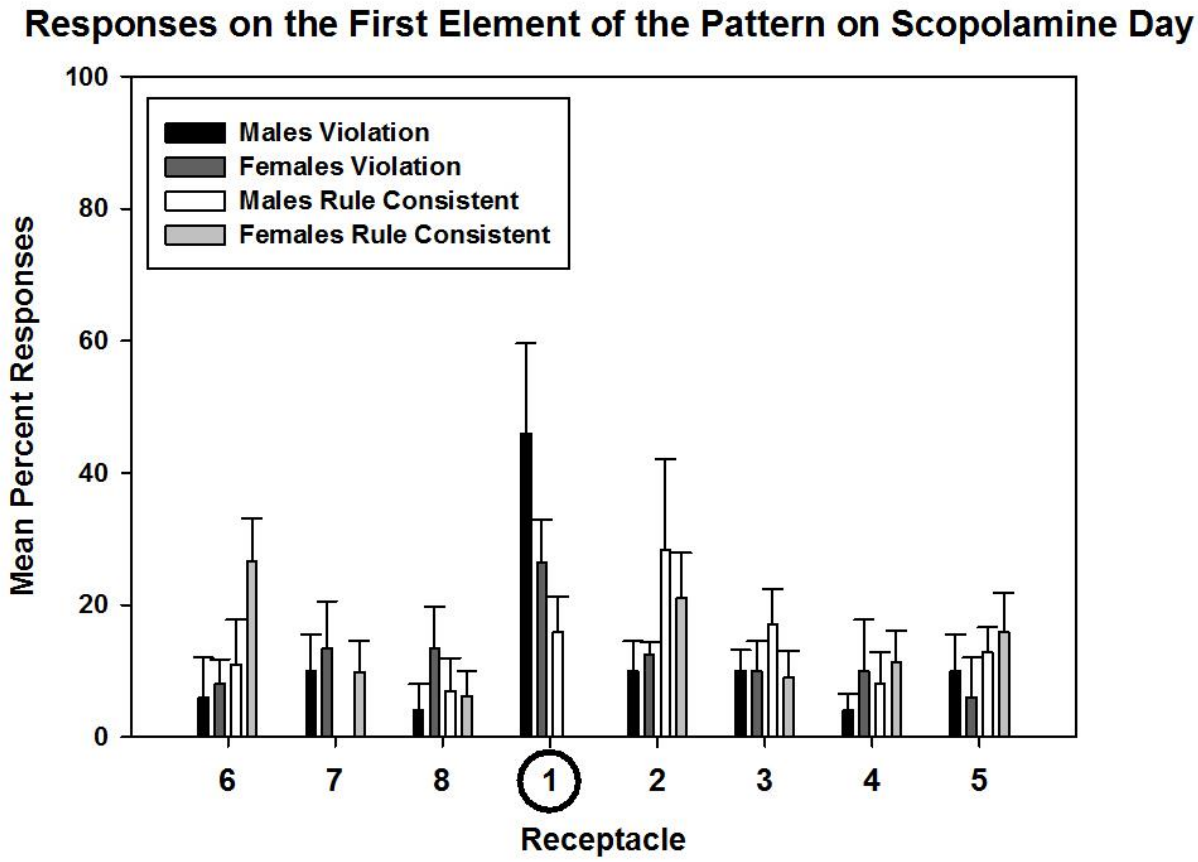

Figure 9. Rat responses on the first element of the 24-element serial pattern on the day of scopolamine administration. An assessment of the distribution of errors did not reveal any between group differences. Correct responses are made at receptacle 1 (shown in the circle).

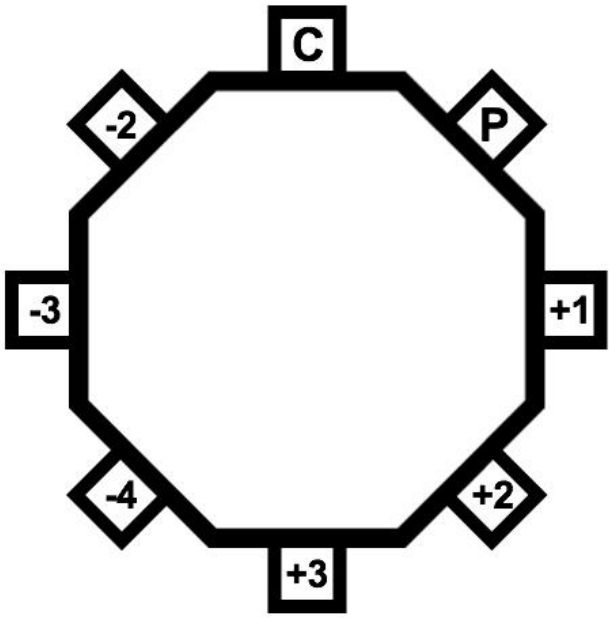

Figure 10. Chunk-boundary analysis spatial layout. “P” represents the position of the last correct element of the pattern and also represents a perseveration, or making a response at the same location as the last correct location. The “C” represents the correct chunk-boundary response location. To make a correct response, rats must respond at the receptacle to the left. “+1”, “+2”, and “+3” all represent rats moving forward one, two, or three receptacles respectively. Going backwards from “P”, rats that make “-2”, “-3”, or “-4” receptacle responses are moving backwards two, three, or four receptacles respectively.

	Percentage of Chunk-Boundary Responses Relative to Correct Receptacle															
	-4		-3		-2		Correct / -1		P*		Within-Chunk / +1		+2		+3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Rule Consistent	5.32	0.96	3.11	1.04	6.64	1.27	15.21	4.54	21.49	2.69	23.82	2.19	14.39	2.56	8.78	2.10
Violation	2.89	0.88	4.87	0.95	6.50	1.16	22.95	3.69	17.10	2.46	21.36	2.00	15.39	2.34	7.87	1.92

Table 1. In relation to Figure 10 – a breakdown of the types of responses made relative to the correct chunk-boundary receptacle. Responses on each chunk-boundary across all patterns were categorized based on spatial position relative to the correct response to analyze a possible strategy rats employed in making chunk-boundary responses on the day of scopolamine administration when a novel chunk was added.